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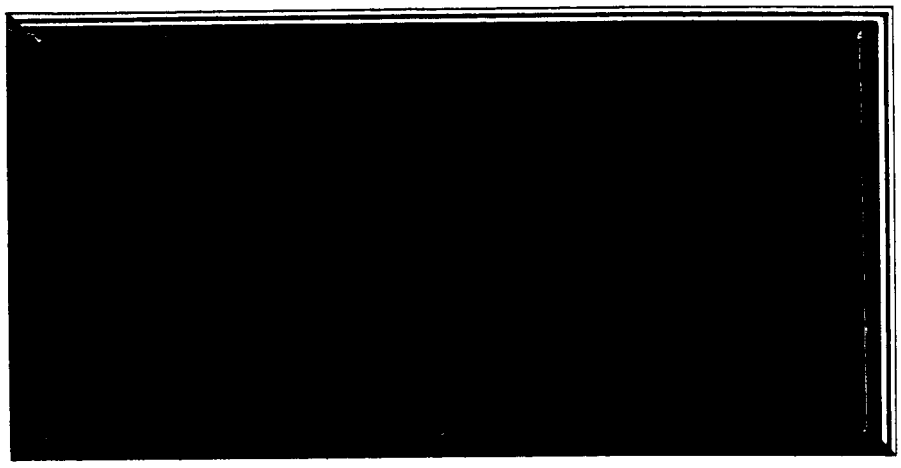

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A STUDY OF THE COLLECTION AND PRESERVATION OF BIOLOGICAL SPECIMENS DURING SPACE FLIGHT FOR POST-FLIGHT ANALYSIS

FINAL REPORT
CONTRACT No. NASW 1562

PREPARED FOR THE
NATIONAL AERONAUTICS & SPACE ADMINISTRATION
BY THE
BIOASTRONAUTICS SECTION
MANNED ORBITING LABORATORY DEPT.
MISSILE & SPACE DIVISION
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NOTICE

PREFACE

This Study Report was prepared by the Missile and Space Division of the General Electric Company for the Office of Manned Space Flight, National Aeronautics and Space Administration, Washington, D. C., under Contract NASW-1562. The Technical Monitors were Dr. Sherman P. Vinograd of NASA Headquarters and Dr. Elliot Harris of the Manned Spacecraft Center, Houston. Contributors to this report were R. C. Bateson, M. H. Bengson, J. W. Boyd, C. A. Carlson, H. Esten, and R. W. Murray.

Dr. T. G. G. Wilson, Assistant Professor of Biochemistry, Temple University Medical School, Staff Biochemist of the Temple Cardiovascular Clinical Research Center and consultant to the General Electric Company, participated in the study and has reviewed this report. Selection of many of the analyses in Section 3 was based on results obtained in his laboratory.

A. Schutzbank and W. D. Isley coordinated the art work and publication of this study.

Since the report which follows is the result of a four months study, no claim to completeness can be made; rather, a detailed survey of a complex problem has been made in an attempt to define some limits of what is known and what should be known for the successful performance of biological experiments in an orbiting laboratory.

Fred W. Thomae, Jr.
Program Manager

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SECTION 1
SUMMARY

This four-month study, Collection and Preservation of Biological Specimens During Space Flight for Post-Flight Analysis, performed under Contract NASW-1562, has included consideration of both the biological and engineering aspects of collection and preservation.

The philosophy underlying the approach taken by the General Electric Company is stated in Section 2. The guiding motive in this approach was to try to assure that analytical results would yield the best data possible in terms of physiological significance, accuracy, precision, and sensitivity. The need for good physiological data is self-evident: extended weightlessness may result in changes in the physiology of astronauts which may be deleterious either temporarily or permanently. Little information is available in regard to physiological changes which may take place in the course of space flight. All that we know is the result of before and after studies, for the most part, for the relatively short periods of the Gemini missions. Another consideration which has guided GE's approach has been the safety of the astronauts, who should not be exposed to hazardous chemicals and procedures.

Analytical methods for constituents of blood, urine, feces, and sweat are described in Section 3. These methods were chosen with a view to eliciting the most precise and accurate data possible from samples preserved during experiments planned by NASA. The question of possible preservation methods also played a role in the choice of techniques because some methods could not be used on preserved specimens; especially in the case of whole blood, in-flight determinations may have to be made since no adequate means of preservation was found. Another factor in the choice of analytical methods was their degree of acceptance and use by research and clinical laboratories. Some techniques which may yield excellent results have not as yet come into general acceptance and, because of this and intrinsic difficulty, their use may be limited to a few research institutions. The techniques chosen are summarized in Tables 3-1, 3-2, 3-3, 3-4, and 3-5.

Sampling and preservation of microorganisms are treated separately in Section 4 because so much of the methodology needs firmer definition, and the quantitation of microbiological data still eludes us. The problems of culturing microorganisms during flight and various techniques of preserving them for post-flight analysis are considered at length. Various old and new methods of sampling are also considered; development of new, and modification of currently used techniques are recommended. Methods of analysis and identification of microorganisms are also discussed. Automatic means of identification, such as gas chromatography, mass spectrometry, infrared spectroscopy, and fluorescent antibody technique which are in the development stage and may be available for routine use in the future are considered.

The engineering and biological aspects of preservation techniques are discussed in Section 5. Based on system weight and state of development, the order of engineering preference for biological sample preservation is rated "best to worst": chemical, refrigeration (chilling), freezing, vacuum distillation, lyophilization, and adsorption-ion exchange. A best approach is selected for each, and the weight, power, and volume penalties are discussed at length. The biological problems involved are considered separately for blood, urine, feces, and sweat. Feces and sweat appear to be the easiest to preserve reliably. Urine presents more difficulties, but most can be solved by several approaches. Blood, however, presents extremely difficult problems with respect to the preservation of some of its constituents. The only solution to some of these at present appears to be in-flight analysis. Other problems have no ready solution but would require laboratory developmental work. All preservation choices require experimental verification and establishment of standard base-lines for any changes in constituents which might occur as a result of a preservation method.

Collection and handling of the samples are discussed in Section 6. Each biological material is discussed separately, and approaches which take into account the absence of gravity and the necessity for scrupulous avoidance of spillage are presented. Several approaches are possible for each material and are described in detail. Some of these make use of designs

already developed by the General Electric Company. Other concepts were developed during the course of this study. Besides devices for collection and storage of the materials themselves, the need for an on-board centrifuge and washing machine is pointed out and preliminary concepts for these devices are shown. Sample containers for holding liquid bacteriological culture media, which permit the addition of samples, are discussed as are storage and transporting modules for all samples. A final sub-section treats the problem of trash disposal and describes a suggested approach.

Sampling regimens are presented in Section 7. Included are schemes for accommodating all the clinical evaluations listed in Appendix A as well as others which would provide a stepwise approach. Sample sizes have been determined so as to allow analyses in duplicate for the constituents of interest. Volume, weight, and power penalties for storing samples by the method of choice are also listed. In addition, clinical evaluations listed in Appendix A, plus others of possible interest have been ranked in groups on the basis of physiological importance and feasibility of performance. Volume, weight, and power penalties for these groups have been determined for the various preservation methods considered. The need for assessment of the relative importance of tests and final priority assignments based on a trade-off between priority assignment by scientific merit and feasibility ranking (by flight schedules, flight qualified hardware lead time, and crew skills required) is pointed out. The problem of physiological importance and feasibility of performance has also led to a tabulation of tests listed in Appendix A which could be omitted from the preservation regimen.

An outline of a training program for astronauts to prepare them for carrying out the procedures necessary for collecting, handling, and preserving biological specimens is described in Section 8. There are two approaches taken depending on whether a flight surgeon is a member of the crew. Also included is an estimate of clock-hours of classroom attendance needed to train a crew to the necessary level of proficiency.

Conclusions of this study and recommendations for future work are included in Section 9.

SECTION 2
INTRODUCTION

This section is a potpourri of all the things that established our philosophy or rationale of approach to the problems involved with collection and preservation of biological specimens in orbit for the purpose of performing post-flight analyses. The overriding consideration throughout this study was to do everything possible to ensure that analyses of the preserved samples would result in data of the highest quality. Figure 2-1 shows the collection to storage sequence.

2.1 GENERAL

The few bits of data extant concerning the physiological changes resulting from exposure to the reduced gravity or "weightlessness" (actually on the order of 10^{-4} or 10^{-5} g) which prevails during space flight are mostly pre- and post-flight measurements. A loss of calcium, attributed to bone demineralization, and a reduction in RBC mass have been noted in the astronauts. But little data is yet available which shows the pattern of these changes, nor the rates at which they occur. If space flights are to be safely extended in duration, it would seem imperative to derive from each flight the maximum amount of biological data possible. Aside from the humane reasons for bringing back a crew unharmed, there are also compelling arguments from the standpoint of costs. The selection and training of space vehicle crew members is expensive. For an exceedingly small fraction of the total mission cost, considerable physiological data can be obtained which would enable those responsible for mission success to adopt appropriate design features to ameliorate the effects of a radical environmental change. The physiological data are necessary for the formulation of design criteria, for example, of devices which could be used as countermeasures for weightlessness.

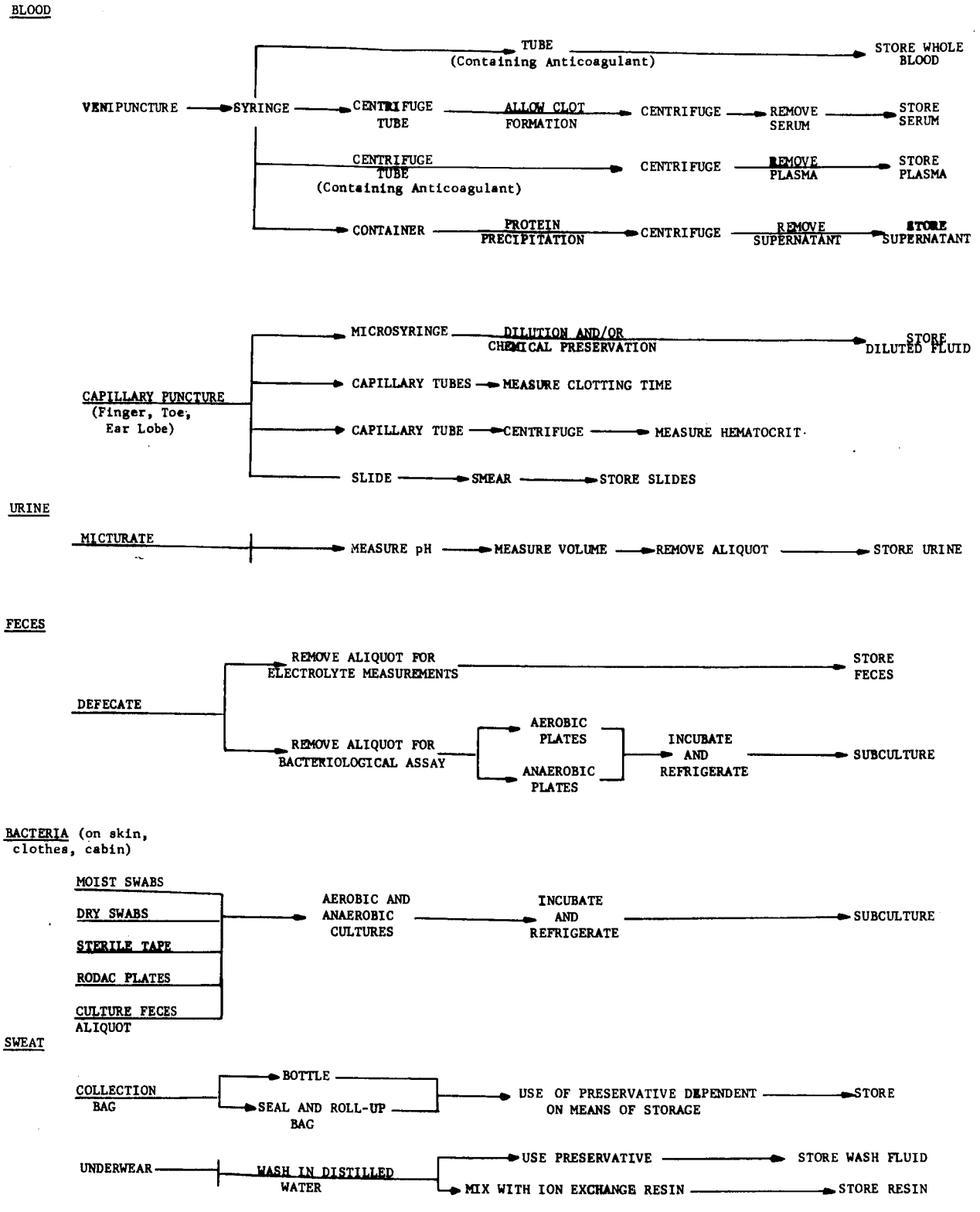

Figure 2-1. Collection to Storage Sequence

There is, as yet, no data to substantiate the explanations offered by various medical personnel regarding the orthostatic hypotension, fluid loss or decrease in RBC mass noted in the Gemini astronauts. Furthermore, the absence of perceivable permanent physiological damage observed in the Gemini astronauts after a 14-day flight is no guarantee that a 30, 180, or 600-day flight will not result in irreversible physiological impairment of crew members. Even more important, from an operational standpoint, is the virtual lack of knowledge concerning the relationship of physiological changes to sustained operational effectiveness of flight personnel.

Any decrements in performance decrease the probability of mission success. It would seem logical to include measurements on man as part of the overall mission planning to insure the success of longer future flights. A large number of measurements are made, during flight, on subsystems and even certain components of the space vehicle. Only by making physiological measurements on man, who is, after all, the major subsystem, can we determine the best space vehicle design for optimum utilization of the capabilities of man.

2.2 SAFETY

To fully utilize man we must, first of all, protect him from known hazards. The recent tragedies at Cape Kennedy and Brooks AFB have underscored the need for a continual watch for real or potential hazards to the safety of the crew. With a complex orbiting laboratory in the planning stages, now is the time to factor in preventive measures. Collection and preservation of samples may seem rather prosaic and for feces, sweat, and urine, it is. Blood collection, however, does pose a safety hazard. The contamination of a syringe due to faulty sterilization or sloppy technique has been responsible for transmission of hepatitis and other infections. Inadequate training can result in injection of air emboli into a vein by an improperly trained person. This, and other reasons, led to the design of the vacuum tubes described in Section 6, which are similar to those used clinically.

Another hazard in the orbiting laboratory will be the presence of large numbers of bacterial cultures on-board. Since subculturing will probably be necessary, the crew must be inculcated with necessity of using aseptic technique. During training, the crew should be made thoroughly aware that all bacteria are potential pathogens. Changes in the indigenous microflora of the crew will occur and resistance to a given organism, cultured a month previous, may be lost. The best way to prevent accidents of this kind will be to include a biologist, experienced in handling microorganisms, in the crew.

Some samples may be preserved with chemicals. Again, the crew should be well indoctrinated in the handling of the chemicals used. Sample containers, with prepacked aliquots of preservatives lessen the problem, but the containers must be handled with care.

Substitutes for other chemicals which are used routinely in ground laboratories may have to be found. An ethyl alcohol swab usually precedes use of a syringe or lancet. Blood slides may have to be simply air dried and stored rather than use a stain dissolved in ethanol or some other volatile solvent.

If analyses are to be done on-board, the analytical methods must be chosen so as to exclude reagents which are hazardous. During the trade-off, planned by NASA, between on-board and post-flight analyses this factor should be weighted heavily.

Safety is an ever present problem in any manned space flight. Sacrifices to other penalties in a trade-off are to be preferred whenever possible to ensure the safety of astronauts. Many of the techniques necessary for collection and preservation of biological specimens during space flight are hazardous to some extent.

In order to do certain analyses at all, venipuncture may be unavoidable. The use of chemical preservatives which are toxic may also be necessary. If lyophilization or vacuum distillation is employed, space vacuum will have to be tapped with the consequent possibility of venting cabin atmosphere in the event of a malfunction.

Because of the need for information concerning changes in biological variables, during extended space flight, a certain amount of risk must be tolerated. By means of adequate training of the astronauts and providing as many built-in safety factors in procedures and equipment as possible, the risk can be reduced to a minimum.

2.3 WHY

The raison d'etre for making measurements of a variety of constituents of blood, urine, feces, and sweat lies in the need for, and present lack of, data regarding the physiological changes occurring during the adaptation of man to the space environment.

The major environmental parameter which is changed during orbital space flight is gravity. When an organism is subjected to a change in environment, it has been shown experimentally that homeostatic mechanisms are brought into play as the organism attempts to adapt. A change in environment can be considered a stimulus which elicits an overall response in the form of adaptation to the changed environment.

Antedating Cannon's concept of homeostasis is the principle of Le Chatelier (which may be considered a corollary to the second law of thermodynamics), i.e., a stress upon a system at equilibrium will result in a change which tends to reduce the stress; thus the system equilibrium will shift in a manner such that the original condition is approached.

Man has evolved under one-g conditions. When man is suddenly thrust into an environment with no "g", gravity dependent mechanisms have lost their point of reference. Furthermore, when a one-g load is removed, such as from the cardiovascular system, mechanisms of adaptation are brought into play. Physiologists have studied the effects of increases and decreases in temperature, atmospheric pressure, partial pressures of O_2 and CO_2, light intensity, sound level, increased "g", and several combinations of these. Now there is the opportunity to design experiments for an environment which does not contain gravity as a parameter. From a scientific standpoint, then, we say, why not ?

With both scientific and military applications of space stations looming on the horizon, a very practical answer to "why?" becomes apparent. Early generation space station crews may not be rotated frequently. Manned flights to Mars are predicated well before the turn of the century. Physiological data must be obtained, if vehicles and equipments are to be designed such that man can fully utilize his capabilities as an observer and experimenter.

Physiological and biochemical data on the course of man's adaptation to weightlessness can only be gotten by using man as an experimental subject while he is exposed to weightlessness. Physiological adaptation is usually a slow process. By frequent periodic measurements, the rates of the various changes associated with adaptation of the whole organism can be ascertained.

2.4 MEASUREMENT CRITERIA

Sampling and collecting of biological specimens during flight is certainly feasible. Some of the analyses will, of necessity, have to be done on-board. Whether a major fraction will be done on-board has not yet been determined. Aids in establishing which tests should be done on-board would be standardized methods and experimentally determined standard curves for both fresh and preserved samples.

However, regardless of which measurements are made on-board and which on the ground post-flight, each measurement and each analysis should meet these criteria:

a. Physiologically significant

b. Accurate

c. Precise

d. Sensitive

By physiological significance of a measurement, we mean that a measurement not only yields useful information regarding some specific physiological variable, but also information which cannot be readily obtained from another measurement. The physiological significance of any given measurement is, admittedly, difficult to determine. Yet, the sampling of subjective opinions of a number of physiologists and clinicians has shown considerable agreement on the rank order of importance of groups of measurements (Section 7) and some agreement on the order within these groups.

In determining the rank order of a number of measurements, based upon physiological significance alone, the question which should be asked by each individual participating in the ranking is: "If I could make only one measurement, which one would I make in order to obtain the most information regarding the physiological status of the experimental subject?" As the list of desired measurements shrinks with each iteration of the question, a rank order is obtained.

Accuracy is defined as the difference between a measured value and the true value of some variable. It is often very difficult to determine true values, however, and in clinical tests for blood, sweat, urine, and feces, it is frequently impossible. Determination of accuracy usually consists of comparing results using a given method with those obtained using a reference method which is generally accepted. When evaluating new methods, a technique sometimes employed is to add a known amount of the substance of interest to a test sample and determine how much is recovered.

Precision is the variation of results when a sample is measured over and over again; a synonym for precision is reproducibility. This is a major source of error in doing the clinical tests described herein. The precision noted on a number of test procedures refers to error when the test is repeated by the same person in the same laboratory within a short period of time. But when precision is measured between different people in different laboratories, the results may be so bad that significant differences in test values would not be able to be detected.

Sensitivity is defined as the minimum quantity of a substance which can be detected by a given analytical method for that substance. Highly sensitive methods are, of course, desirable. Substances which occur in very low concentrations require analytical methods of high sensitivity, but substances which are found in high concentrations do not require highly sensitive techniques. It must be remembered, however, that the sensitivity of a method is a determining factor in being able to distinguish between concentrations which only vary slightly. In most cases of this kind, the precision of a method will be the final limiting factor unless sensitivity is very poor.

During the course of this study we have attempted to select analytical methods, for each of the measurements in Appendix A, with the attributes of accuracy, precision, sensitivity, and also the preservation methods which have the fewest effects on these attributes.

Now that this information is in hand, the trade-off of on-board vs. post-flight measurements can be performed with the option of adding physiological significance as a weighting factor.

SECTION 3
ANALYTICAL METHODS

The analytical methods chosen for the assays listed in Appendix A, furnished by NASA for this study, are described in this section.

Dr. T.G.G. Wilson of the Cardiovascular Clinical Research Center of Temple University Medical Center acted as a consultant on this as well as other aspects of this contract effort. He reviewed the chosen analytical methods in the light of current clinical practice. At Temple, he reviews all new biochemical techniques to ascertain their applicability to the problems of cardiovascular research.

3.1 BLOOD

A considerable body of literature pertaining to clinical biochemistry was reviewed. References were obtained by computer search, from bibliographies in recent reference and text books, Biological Abstracts, Chemical Abstracts, and Index Medicus. In addition, a number of contacts with individuals working in specialized areas were made in an effort to isolate and identify the best method of performing a particular analysis.

BLOOD CONSTITUENT ANALYSES

The guidelines used for selection of the best method for a given analysis on a preserved sample of whole blood, plasma, serum, or the formed elements were chosen with scant regard to the man-hours and capital equipment involved. Rather, accuracy, precision, and sensitivity as well as acceptance of the method by reputable investigators were used as criteria in deciding upon a method of choice. An additional criterion was that of applicability to preserved samples. In the main, analyses which adhered to our criteria were also those methods which tended to be lowest in overall cost of performance.

Analyses for elements, inorganic radicals, and the simpler organic compounds were easier to select, particularly since the bulk of these determinations are done on serum. However, when the analyses listed in appendix A for whole blood or plasma are considered, it becomes readily apparent that while sensitivity may be retained simply by means of definition, precision is utterly appalling, and accuracy, in most cases, is conspicuously absent because of the great difficulties in determining standards. What becomes evident, after fruitless hours of literature searches, is that a dichotomy of analytical methods exists with highly accurate, very precise, and extremely sensitive quantitative methods in one group and essentially qualitative methods in the other, with very little between. This fact of life is summarized in Tables 3-1, 3-2, and 3-3.

The methods chosen for qualitative measures (e.g. WBC differential) and the more ephemeral attributes of blood (e.g. platelet adhesiveness) are the best available, but unfortunately, the best available are none too good.

When the term "not a quantitative test" is used in this section to describe particular constituent analyses, it means that absolute standards for these constituents are not available and the result of tests can only be considered relative. These tests are empirical; so-called "standard values" are obtained by the assay of a large number of individuals who are considered normal or by pooled samples of serum, plasma, or whole blood from many patients. There are no reference standards available such as those from the National Bureau of Standards for radioisotopes, etc. which could be used to establish the accuracy of the analytical technique. Furthermore, the accuracy of the tests cannot be established in terms of recovery of added substance because known quantities of the constituent are unavailable for addition to samples. Precision is noted, however, in those cases where data was available.

It must be noted that, when a given volume of serum or plasma is required, twice that volume of whole blood must be withdrawn. For example, if 1 ml of serum is needed,

Table 3-1. Blood Constituent Analyses, Whole Blood

Whole Blood Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method	Anticoag. Required
Blood Lactic Acid	Barker & Summerson	1-3 ml	± 5%	95% - 105% recovery	Freezing of precipated solution	None
Hematocrit	Microhematocrit	60-100 μl			In-flight determination	Heparinized capillary tube
Reticulocyte Count	Couting of stained blood smear	0.1 ml		Not Quantitative Methods	Chemical	None
RBC (Total)	Counting in hemocytometer	0.1 ml			Chemical	Citrate
WBC (Total)	Couting in hemocytometer	0.1 ml			Chemical	Citrate
WBC Differential	Counting of stained blood smear	0.1 ml			Chemical	None
RBC Cell Mass	Isotopic dilution tehniques	1 ml			Refrig. or freezing	Any
RBC Survival	Isotopic labeling of red cells	1 ml			Refrig. or freezing	Any

Table 3-1. Blood Constituent Analyses, Whole Blood (Cont)

Whole Blood Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method	Anticoag. Required
Hemoglobin						
Macro	Oxyhemoglobin Method	0.1 ml	± 3%	Abnormal Hemoglob. increase values	Freezing of dilute, hemolyzed sample	None
Micro	Cyanmethemoglobin Method	20 µl	± 3%			
Ultramicro	Cyanmethemoglobin Method	1.5 µl	± 3%			
Platelet Count (Est)	Indirect Counting Method of Dameshek	0.1 ml			Chemical	None
WBC Motility and Phagocytic Activity	Microscopic Observation	20 ml			In-flight alternative or not at all	Heparin NOT Citrate
Platelet Adhesiveness		16 ml		Not Quantitative Methods	In-flight determination	
Karyotyping	Phytohemagglutinin Incubation - Arakaki	0.1-0.2 ml			Technique needs to be developed. In-flight alternative possible	Heparin
Clotting Time	Capillary Tube Method	0.1-0.5 ml			In-flight determination	None
Clot Retraction	Observation of clot retraction	2-3 ml			In-flight determination or not at all	None
Methemoglobin	Evelyn and Malloy	0.1 ml	± 5%		Freezing of dilute, hemolyzed sample	None

Table 3-2. Blood ConstituentAnalyses, Plasma

Plasma Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method	Anticoag. Required
Amino Nitrogen	Folin	1 ml	± 5%	Not highly accurate but results are reproducible an accepted as standard by many labs	Freezing	Any
Fibrinogen	Fibrinogen test - Andersch-Gibson modification	1 ml			Freezing	Oxalate
Fibrinolytic Activity	Stefanini-Dameshek Screening Test	1.5 ml			Freezing	Citrate
Prothrombin Activity	Specific determination - Owren	0.2 ml		Not Quantitative	Freezing	Oxalate
Plasma Thromoplastic Comp. (PTC)	Assay for Factor IX	1 ml		Methods	Freezing	Oxalate
Antihemophilic Globulin (AHG)	Jung	0.1 ml			Freezing	Citrate
Antidiuretic Hormone (ADH)	Bioassay Technique	10 ml (?)			Freezing	Citrate
Adrenocorticotrophic Hormone (ACTH)	Bioassay Technique	2 ml			Freezing	Citrate

Table 3-3. Blood Constituent Analyses, Serum

Serum Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method
Creatine					
Macro	Folin	0.5 ml			Freezing
Micro	Owen et al.	0.2 ml	± 11%		Freezing
Ultramicro	Owen et al.	40 µl	± 9.8%		Freezing
Creatinine					
Macro	Folin and Wu	0.5 ml			Freezing
Micro	Owen et al.	0.2 ml	± 11.0%		
Ultramicro	Owen et al.	40 µl	± 9.8%		
Serum Proteins	Paper Electrophoresis	10 µl	±5 to ± 15%		Freezing
Mucoproteins	O'Brien and Ibbott	0.8 ml	± 15.0%	Recovery is only about 70%	Freezing
Sodium					
Micro	Flame Photometry	0.1 ml	± 1.5 to ± 5%	Highly Accurate	Refrigeration, Freezing
Ultramicro	Flame Photometry	20 µl			
Potassium					
Micro	Flame Photometry	0.1 ml	± 2 to 15%	Highly Accurate	Refrigeration, Freezing
Ultramicro	Flame Photometry	20 µl			
Chlorides					
Macro	Schales and Schales	1.0 ml	± 2%	± 1%	Refrigeration, Freezing
Micro	Schales and Schales	40 µl	± 3%		
Ultramicro	Schales and Schales	10 µl	± 3.6%		

Table 3-3. Blood Constituent Analyses, Serum (Cont)

Serum Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method
Phosphates					
Macro	Fiske and Subbarow	1.0 ml	± 4%	Up to 5% too high values are possible	Freezing
Micro	McDonald and Hall	0.1 ml	± 4.2%		
Ultramicro	Fiske and Subbarow	10 μl	± 6.1%		
Akaline Phosphatase					
Macro	Shinowara et al.	0.4 ml		Presence of inhibitors or activators can lead to spurious values	Freezing
Micro	Bessey et al.	0.1 ml	± 2.9%		
Ultramicro	Bessey et al.	10 μl	± 3.3%		
Calcium					
Macro	Ferro and Ham	2 ml		Errors tend to cancel each other out	Refrigeration, Freezing
Micro	Ferro and Ham	0.5 ml	± 6.0%		
Ultramicro	Diehl and Ellingboe	20 μl	± 4.2%		
Magnesium	Atomic Absorption Spectrophotometry	0.1-0.5 ml	± 2%	Highly Accurate	Refrigeration, Freezing
Manganese	Atomic Absorption Spectrophotometry	0.1-0.5 ml	± 2%	Highly Accurate	Refrigeration, Freezing
Bicarbonate					
Macro	Scribner-Caillouette	1.5 ml			
Ultramicro	Microtitrimetry	10 μl	± 15.8%		Freezing
Zinc	Atomic Absorption Spectrophotometry	0.1-0.5 ml	Better than 5%	± 5%	Refrigeration, Freezing

Table 3-3. Blood Constituent Analyses, Serum (Cont)

Serum Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method
Sulfates	Kleeman, Taborsky and Epstein	1 ml	± 5%	98-100% recoveries	Freezing
Non-Protein Nitrogen (NPN)					
Macro	Folin and Wu	0.5 ml			Freezing
Micro	Folin and Wu	0.1 ml			
Blood Urea Nitrogen (BUN)					
Macro	Xanthydrol Reaction	0.3 ml		Urease is specific but inaccuracies from other sources possible	Freezing
Micro	Urease Method	0.1 ml	± 6.0%		
Ultramico	Urease Method	10μl	± 12.4%		
Uric Acid					
Macro	Henry et al.	0.4 ml	± 5%	Recoveries of 87% to 98% are possible	Freezing
Micro	Henry et al.	0.2 ml			
Ultramicro	Caraway	20 μl	± 7.5%		
Glucose					
Macro	Folin and Wu	0.1 ml	± 3%	Non-specific reduction leads to high values	Freezing
Micro	Marks	0.1 ml	± 4.6%		
Ultramicro	Marks	10 μl	± 11.0%		
Lipids	Turbidimetric Determination	0.1 ml	Not a Quantitative Method		Freezing

Table 3-3. Blood Constituent Analyses, Serum (Cont)

Serum Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method
Bilirubin					
Macro	Diazo method of Evelyn-Malloy	1.0 ml		Many sources of error which are hard to avoid. Reproducibility in normal (non-elevated) range is poor.	Freezing
Micro	Diazo method of Evelyn-Malloy	0.1-0.2 ml	± 6.4%		
Ultramicro	Diazo method of Evelyn-Malloy	80 μl	± 11.0%		
Protein Bound Iodine (PBI					
Macro	Chloric Acid Oxidation-Zak	2 ml	± 6%	Better than 95% recovery	Freezing
Micro	Sanz et al.	50 μl			
Catecholamines	Fluorometric Determination	10 ml	Good	75% recovery	Freezing
Thyroxine	Column Chromatography	2-3 ml	± 10%	Better than 90% recovery but all iodo-thyronines are measured.	Freezing
Thyroxine Binding Prealbumin (TBPA)	Starch Gel-Electro-Phoresis or Protein Assay	2 ml		Not Quantitative Methods	Freezing
Plasma Volume	Isotopic dilution using ^{125}RISA	1 ml			Refrigeration, Freezing.

Table 3-3. Blood Constituent Analyses, Serum (Cont)

Serum Constituent	Analytical Method	Volume Required	Precision	Accuracy	Preservation Method
Immunoglobulins	Paper Electrophoresis and specific immunological tests	5-10 ml		Not Quantitative Methods	Freezing
LDH Isozymes	Starch gel electrophoresis and enzyme activity tests	0.1 ml	± 6%		Freezing
Immune (Anti) Bodies	Immune electrophoresis Precipitin Test, Complement Fixation Test Agglutination	1.0 ml *			Freezing
Transferrins	Iron-Binding Capacity Determination	0.5-1.0 ml	± 5%		Freezing

* See Text

2 ml of whole blood is withdrawn. The reason for this is that close to half of whole blood is composed of cellular material (erythrocytes, leukocytes, and platelets).

1. Creatine: Method of Folin; Owen et al.

A protein-free blood filtrate is hydrolyzed with acid in an autoclave, is then treated with an alkaline picrate solution and compared spectrophotometrically with a standard solution treated in the same way. The picrate is reduced to picramate which is red in an alkaline solution, by the creatinine produced by hydrolysis. The creatinine content of an unhydrolyzed sample must then be subtracted.

Fluid: Serum
Volume: 0.5 ml Macro
0.2 ml Micro
40 micro 1. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:		±11.0%	±9.8%
Accuracy:			

Method of Preservation: Freezing and storage at -20°C. Change in level of creatine at this temperature should not be significant over a period of 6 months, but laboratory study would have to establish this. At room temperature, some conversion of creatinine to creatine occurs in blood.
Problems: None apparent.

References: 1-4, 53, 62-68.

2. Creatinine: Method of Folin and Wu; Owen et al.

Same reaction used as that for creatine except it is done on a nonhyrolyzed filtrate.

Fluid: Serum
Volume: 0.5 ml Macro
0.2 ml Micro
40 micro 1. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:		±11.0%	± 9.8%
Accuracy:			

Preservation: Freezing and storage at -20°C. Same limitations as for creatine.
Problems: None apparent.

References: 1-4, 53, 62-68.

3. Serum Proteins: Method: Paper Electrophoresis

A quantity of serum is placed on a paper strip, wetted with buffer and exposed to an electric field. Proteins migrate to the different poles at rates dependent upon their size and electric charge. They are then stained and fixed. The paper strips are cut up, separating the components. Stain is eluted from each portion and measured photometrically. This yields a measure of specific protein concentrations.

Fluid: Serum
Volume: 10 micro 1.
Sensitivity:
Precision: ±5 to ±15%
Accuracy:
Preservation: Freezing and storage of separated serum at -20°C. Serum is believed to be stable for at least 6 months under these conditions for electrophoresis.
Problems: Hemolysis should be avoided as it leads to errors in technique.

References: 1, 53.

4. Mucoproteins: Method of O'Brien and Ibbott, Modified from Winzler et al.

Separate tests can be run on mucoprotein tyrosine, carbohydrate and protein employing specific colorimetric reactions which are then measured spectrophotometrically.

Fluid: Serum
Volume: 0.8 ml
Sensitivity:
Precision: ±15%
Accuracy: Recovery is only about 70%.
Preservation: Freezing and storage at -20°C of separated serum. There is little information available on the effect of this type of storage upon this parameter. It would seem advisable to study this effect although an educated guess would be that such storage would produce little change.
Problems: None apparent.

References: 2, 53, 69

5. Sodium: Method: Flame Photometry

The intensity of a specific wavelength of the sodium atomic emission spectrum in a diluted, atomized sample is a measure of the sodium concentration.

Fluid: Serum
Volume: 0.1 ml Micro
20 micro 1. Ultramicro
Sensitivity: Depends on type of photometer used. Perkin-Elmer, Model 146 has a sensitivity of 4.3×10^{-7} meq/1. while another instrument has one of 2×10^{-2} meq/1.
Precision: ± 1.5% (within a single laboratory)
± 5% (between different laboratories)
Accuracy: Highly accurate
Preservation: Any method will do, but, to prevent degradation due to autolysis, it would probably be well to refrigerate or freeze samples of serum for this test parameter, too.
Problems: It is necessary to separate serum from blood cells immediately upon completion of clotting after withdrawal of sample because otherwise ion exchange between cells and serum will alter the original values. Anticoagulants which contain sodium must be avoided.

References: 1, 2, 3, 5, 53.

6. Potassium: Method: Flame Photometry

The intensity of a specific wavelength of the potassium atomic emission spectrum in a diluted, atomized sample, is a measure of the potassium concentration.

Fluid: Serum
Volume: 0.1 ml Micro
20 micro 1. Ultramicro
Sensitivity: Depends on type of photometer used.
Precision: ±2.0% (within a single laboratory)
±15% (between different laboratories)
Accuracy: Highly accurate
Preservation: See Sodium.
Problems: See Sodium. Hemolysis is a particularly serious problem here because of preponderance of K^+ in the cell (only slight hemolysis markedly increases the serum K^+). Anticoagulants containing potassium must be avoided.

References: 1, 2, 3, 5, 53.

7. Chlorides: Method of Schales and Schales as modified in Ref. 1 and Ref. 2

Mercurimetric technique involves addition of mercuric ions to a sample which combine with chloride ions until there is an excess of mercuric ions which combine with diphenylcarbazone producing a purple color (end point).

Fluid: Serum
Volume: 1 ml Macro
40 micro 1. Micro
10 micro 1. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:	± 2%	±3%	± 3.6%
Accuracy:	± 1%		

Preservation: To avoid degradation, refrigeration or freezing should be used.
Problems: Serum should be separated from cells immediately after withdrawal and clotting to prevent change in values due to ion exchange between fluid and cellular components.

References: 1, 2, 53, 70.

8. Phosphates (Inorganic Phosphorus): Method of Fiske and Subbarow (Macro and Ultramicro); method of McDonald and Hall (Micro)

These techniques involve the reaction of inorganic phosphate with molybdic acid or ammonium molybdate which produces a phosphomolybdate complex. The complex is then reduced to a colored compound by the use of a variety of reagents, and absorbance is measured spectrophotometrically.

Fluid: Serum
Volume: 1.0 ml Macro
0.1 ml Micro
10 micro 1. Ultramicro

Sensitivity:
Precision: ±4% - Macro ± 4.2% - Micro ± 6.1% - Ultramicro
Accuracy: Values may be as much as 5% too high due to background absorption.
Preservation: Freezing and storage of separated serum at -20° C. Information concerning change in phosphate level using this technique is unavailable. A laboratory study would be necessary to establish and standardize any change.

Problems: Serum must be separated from cells immediately after withdrawal of blood sample because phosphate level is increased by breakdown of cell organic phosphorus or by hemolysis. This may be a problem in separated serum itself, also. Containers used to collect blood must be free of phosphorus contamination.

References: 1, 2, 3, 53, 71, 72.

9. Alkaline Phosphatase: Method of Shinowara et al. (Macro); Bessey et al. (Micro and Ultramicro)

These methods employ colorimetric reactions for phosphate liberated from organic substrates by the enzyme in serum.

Fluid: Serum
Volume: 0.4 ml Macro
0.1 ml Micro
10 micro 1. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:		± 2.9%	± 3.3%

Accuracy: Presence of inhibitors or activators can lead to spurious values.
Preservation: Freezing and storage of separated serum at 20°C. This is a technique which is suggested, but which will undoubtedly require a laboratory study to standardize changes. See problems.
Problems: Storage of serum at -10°C for 6 months produces significant drops in alkaline phosphatase levels (4). Colder storage temperature may reduce this change but probably will not stop it completely unless cryogenic temperatures are employed. Further problems are the same as those for inorganic phosphate.

References: 2, 3, 4, 53, 72-74.

10. Calcium: Method of Ferro and Ham (Macro and Micro); Diehl and Ellingboe (Ultramicro), modified

The macro and micro method employ precipitation of calcium with chloranilic acid. After washing, the precipitate is then dissolved in EDTA which produces a colorimetric reaction which is compared with standards in a spectrophotometer. The ultramicromethod employs microtitration technique which involves the conversion of a fluorescing reagent to a non-fluorescent end product.

Fluid: Serum
Volume: 2 ml Macro
0.5 ml Micro
20 micro 1. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:		± 6.0%	± 4.2%

Accuracy: Errors seem to cancel each other out, leaving a net result which is highly accurate.
Preservation: Any method will do, but to avoid degradation, refrigeration or freezing of separated serum should probably be employed.
Problems: Serum must be removed from cells after withdrawal of blood sample; the use of anticoagulants which bind calcium must be avoided. Spurious results would otherwise be obtained. Serum should be separated immediately to prevent alteration of values due to exchange with cells.

References: 2, 3, 19, 53, 75-77.

11. Magnesium: Method: Atomic Absorption Spectrophotometry

Light absorbed at the wavelength of the resonance line by the unexcited atoms of an element is measured. A sample is sprayed into a flame to provide a reproducible and clearly defined cloud of atoms, and the absoption of light from a source which emits the line spectra of the element of interest is a measure of its concentration.

Fluid: Serum
Volume: 0.1 - 0.5 ml
Sensitivity: 0.01 μg/ml
Precision: ± 2%
Accuracy: Highly accurate.
Preservation: Any method will do, but refrigeration or freezing should probably be employed to avoid degradation.
Problems: Serum must be separated from cells shortly after withdrawal of sample.

References: 53, 54-58.

12. Manganese: Method: Atomic Absorption Spectrophotometry

Description: See Magnesium.
Fluid: Serum
Volume: 0.1 - 0.5 ml (?)
Sensitivity: 0.05 μg/ml
Precision: ± 2%
Accuracy: Highly accurate.
Preservation: Any method: see Magnesium.
Problems: See Magnesium.

References: 53, 54.

13. Bicarbonate: Method of Scribner-Caillouette (Macro); microtitrimetry (ultramicro)

Both techniques involve acid-base titration to determine the amount of bicarbonate in serum by back-titration after the addition of excess acid.

Fluid: Serum
Volume: 1.5 ml Macro
10 micro 1. Ultramicro

	MACRO	ULTRAMICRO
Sensitivity:		
Precision:		± 15.8%
Accuracy:		

Preservation: Freezing and storage of separated serum at -20°C. Since information is not available on the change of bicarbonate during storage, a laboratory study would be required to obtain standard curves.
Problems: Since bicarbonate is in equilibrium with dissolved carbon dioxide in blood and the pH of blood is one measure of this relationship, the pH of blood should be measured at the time of withdrawal. For the same reason, additives such as anticoagulants which might alter pH should be avoided. Serum should be separated from cells immediately in closed containers and frozen as quickly as possible. Even then, the relationship between bicarbonate remaining after these procedures and the original value would have to be established by a laboratory study before results could be meaningful.

References: 2, 6, 78.

14. Zinc: Method: Atomic Absoprtion Spectrophotometry

Description: See Magnesium.
Fluid: Serum
Volume: 0.1 - 0.5 ml
Sensitivity: 0.002 μg/ml
Precision: Better than ± 5%
Accuracy: ± 5%
Preservation: See Magnesium
Problems: See Magnesium.

References: 53, 54, 59.

15. Sulfates: Method of Kleeman, Taborsky and Epstein, modified

Serum is deproteinized with uranyl acetate. This also removes phosphate. The sulfate in the filtrate is then precipitated as benzidine sulfate, and the bezidine is measured photometrically by reaction with sodium beta-napthoquinone-4-sulfonate.

Fluid: Serum
Volume: 1 ml
Sensitivity:
Precision: ± 5%
Accuracy: Recoveries of 98-100% have been obtained.
Preservation: Freezing and storage of separated serum at -20°C. The effect of this type of preservation would have to be investigated definitively.
Problems: Sulfate level in serum (unless frozen) changes due to degradation of organic sulfates.

References: 53, 100.

16. Non-Protein Nitrogen (NPN): Method of Folin and Wu

This technique employs a micro-Kjeldahl procedure for the determination of nitrogen upon a protein-free filtrate of serum.

Fluid: Serum
Volume: 0.5 ml Macro
0.1 ml Micro
Sensitivity:
Precision:
Accuracy:
Preservation: Freezing and storage of separated serum at -20°C. See problems.
Problems: Information exists showing that NPN changes significantly when stored at -10°C for six months (4). For this reason, changes occurring at the lower temperature would have to be studied in a laboratory investigation.

References: 1, 3, 4, 62, 79.

17. Blood Urea Nitrogen (BUN): Method: Xanthydrol Reaction (Macro); Urease Method (Micro and Ultramicro)

The xanthydrol reaction involves precipitation of urea with xanthydrol resulting in dixanthydryl urea which is then assayed. The urease method employs the enzyme to produce ammonia from urea, which is then measured by nesslerization.

Fluid: Serum
Volume: 0.3 ml Macro
0.1 ml Micro
10 micro l. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:		± 6.0%	± 12.4%

Urease is a specific enzyme, but inaccuracies may arise due to other steps of the procedure. A purified enzyme preparation must be used.

Preservation: Freezing and storage of separated serum at -20°C. Changes occurring, if any, during preservation would have to be quantitated by laboratory study. Little change was observed in the one study available (4), but this study did not start with fresh serum.

Proflems: Anticoagulants containing ammonia produce spurious results. Mercury contamination of containers must be avoided.

References: 1, 2, 3, 4, 80.

18. Uric Acid: Method of Henry et al. (Macro and Micro); Method of Caraway (Ultramicro)

Both methods employ the reduction of hexavalent tungsten by uric acid to a lower valence with the formation of a blue color which is measured spectrophotometrically.

Fluid: Serum

Volume: 0.4 ml Macro
0.2 ml Micro
20 micro 1. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:	± 5%		± 7.5%

Accuracy: Recoveries of 87% to 98% have been reported.

Preservation: Freezing and storage of separated serum at -20°C. The effect of such storage needs investigation, but on one test in the literature, there was little change in 6 months at -10°C. The serum used, however, was not fresh (4).

Problems: Separation of serum from whole blood must be immediate because colored products may be produced by whole blood contamination.

References: 2, 3, 4, 53, 81, 82.

19. Glucose Tolerance Test:

This test involves the measurement of glucose concentration in blood (serum can be used) before and at several timed intervals after the administration of a known quantity of glucose to a fasting subject. Urine can also be collected and its glucose concentration determined. Four or five aliquots of blood are usually withdrawn.

19a. Glucose: Method of Folin and Wu (Macro); of Marks, modified (Micro and Ultramicro)

The macro method employs the reaction of glucose with an alkaline copper solution to produce cuprous oxide from cupric hydroxide. The cuprous oxide reduces phosphomolybdic acid to phosphomolybdous acid which is blue. Comparison with a glucose standard is then made spectrophotometrically. The micro and ultramicro method employs a specific sequence of enzymatic reactions for glucose resulting in a colored product. This enzymatic procedure avoids high estimates due to non-specific reducing substances in blood.

Fluid: Serum
Volume: 0.1 ml Macro
0.1 ml Micro
10 micro l. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:	± 3%	± 4.6%	± 11.0%

Accuracy: Non-specific reduction leads to values which are too high in the macro method, but are used standardly by many laboratories.
Preservation: Freezing and storage of separated serum at -20°C.
Problems: Serum must be separated immediately from cells since erythrocytes will utilize the sugar resulting in spuriously reduced readings. The effect of storage must be investigated. Storage for 6 months of serum at -10°C resulted in significantly reduced values. Standardization of such a decrease would have to be effected. Blood preservatives containing glucose must be avoided.

References: 1, 2, 3, 4, 53, 83-86.

20. Fat Tolerance Test:

A fasting subject who has had an essentially fat-free supper the night before is fed a fat-free breakfast plus heavy cream containing 60 grams of butter fat. Samples of venous blood are taken before this meal and at hourly intervals beginning one hour after the meal was begun and continuing for 8 hours.

20a. Lipids: Turbidimetric Determination

The turbidity of serum samples whose lipid has been precipitated with phenol at high salt concentration is determined photometrically.

Fluid: Serum
Volume: 0.1 ml

Sensitivity:)
Precision:) not a quantitative measurement
Accuracy:)
Preservation: Freezing and storage of separated serum at -20°C. The effect of this type of storage on lipid content of serum is unknown and should be evaluated in a laboratory study.
Problems: This test is not in widespread use in clinical laboratories. It is possible that fat tolerance is decreased under stress.

References:

21. Amino Nitrogen: Method of Folin

The amino acid nitrogen in plasma is determined by photometric measurement of the color produced by reaction with sodium beta-naphthoquinone 4-sulfonate.

Fluid: Plasma
Volume: 1 ml
Sensitivity:
Precision: ± 5%
Accuracy: Technique is not highly accurate, but leads to reproducible results accepted as standard by many laboratories. No technique is absolutely accurate. Uric acid and sulfonamides produce spurious increases in levels.
Preservation: Freezing and storage of separated plasma at -20°C. Anticoagulants must be used to prevent clotting.
Problems: Contact with red cells may be deleterious so immediate separation is advisable. The effect of storage on the levels of amino nitrogen is now known. Laboratory study of the problem would be necessary. Serum cannot be used because the clotting process produces an increase of 10 to 40% in the alpha-amino nitrogen level.

References: 1, 53.

22. Blood Lactic Acid: Method of Barker and Summerson, Modified

Lactic acid when heated with concentrated sulfuric acid forms acetaldehyde which is then reacted with p-phenyl-phenol to form a fairly stable colored compound which is proportional to the lactate concentration. This compound is measured spectrophotometrically.

Fluid: Whole Blood
Volume: 1 - 3 ml
Sensitivity:
Precision: ± 5%

Accuracy: ± 5% (based on recoveries of lactate added to blood)
Preservation: Precipitated solution may be able to be stored by freezing. See Problems.
Problems: The protein in each sample must be precipitated immediately with trichloroacetic acid. If this is not done, the lactate concentration of blood changes very rapidly. Whether or not this precipitated solution can be stored by freezing or some other method would have to be investigated by a laboratory study. Contamination by skin lactic acid must be avoided.

References: 2, 6, 7, 53, 87.

23. Bilirubin: Diazo Method of Evelyn-Malloy

Diazotized sulfanilic acid is coupled with bilirubin to produce a colored compound called azobilirubin which is measured spectrophotometrically.

Fluid: Serum
Volume: 1.0 ml Macro
0.1 - 0.2 ml Micro
80 micro 1. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:		± 6.4%	± 11.0%

Accuracy: Many sources of error which are hard to avoid. Reproducibility in normal (nonelevated) range is poor.
Preservation: Freezing and storage of separated, non-hemolyzed samples at -20°C.
Problems: Separation of serum from cells must be done immediately to avoid any traces of hemolysis which lead to spurious values. Specimens should be collected when subject is in a post-absorptive state because lipemia causes elevated readings. Samples must be protected against exposure to light which photooxidizes bilirubin. Storage as described will probably produce little change in bilirubin values, but this will have to be verified by laboratory study. Frozen samples stored in the dark are stable for at least 3 months.

References: 1, 2, 3, 4, 53, 88-91.

24. Protein Bound Iodine (PBI): Methods: Chloric Acid Oxidation - Zak, modified (Macro); Sanz et al. (Micro)

The macro method employs digestion of precipitated protein with chloric acid and determination of iodine present by the catalytic reduction of Ce^{+4} to Ce^{+3}. The micro method employs the same catalytic reduction upon ashed samples.

Fluid: Serum
Volume: 2 ml Macro
50 micro 1. Micro
MACRO MICRO
Sensitivity:
Precision: ± 6%
Accuracy: Better than 95% recovery of added thyroxine is possible.
Preservation: Freezing and storage of separated serum at -20°C.
Problems: Contamination of equipment with iodine or mercury leads to spurious values. Exogenous iodine in the subject from a variety of sources may alter results. The effect of storage under these conditions is not known and would have to be studied although this is the routine method used for storing serum for control studies.

References: 1, 2, 53, 92-94.

25. Catecholamines: Method: Fluorometric Determination

Catecholamines are selectively adsorbed onto aluminum oxide. They are then eluted, and the eluate is oxidized to a fluorescent trihydroxyindole derivative. The degree of fluorescence is a measure of the catecholamine concentration.

Fluid: Serum
Volume: 10 ml
Sensitivity: 0.5 - 0.75 μg/1
Precision: Good
Accuracy: 75% recovery
Preservation: Freezing and storage of separated serum at -20°C in the presence of a reducer, e.g. Na metabisulfite.
Problems: The large size of the sample required may be prohibitive. The value of the test is open to question since the levels of catecholamines in peripheral blood represent transitory phenomena. It would be more valuable to study levels in excreted urine collected over longer periods of time.

References: 1, 8, 60, 61.

26. Thyroxine: Method: Column Chromatography

Acidified serum is chromatographed using an ion exchange resin, and thyroxine and other iodothyronines are eluted with acetic acid. The iodine content of the eluate is determined by applications of a ceric-arsenite reaction following aklaline incineration of dried aliquots.

Fluid: Serum
Volume: 2 - 3 ml
Sensitivity:
Precision: ± 10%
Accuracy: All iodothyronines including thyroxine are measured. Recovery of added thyroxine is better than 90%
Preservation: Freezing and storage of separated serum at -20°C. The effect of this type of storage on thyroxine concentrations would have to be established by laboratory study.
Problems: Inorganic iodine contamination does not cause spurious readings with this method, but contaminatior with organic iodine may.

References: 53, 101.

27. Thyroxine Binding Pre-Albumin (TBPA): Method: Starch Gel Electrophoresis or Protein Assay

The protein is first isolated and then estimated by either of the methods mentioned.

Fluid: Serum
Volume: 2 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated serum at -20°C. The effect of this type of storage upon TBPA concentrations would have to be determined by laboratory study.
Problems: See Preservation.

References: 102, 103.

28. Antidiuretic Hormone (ADH): Bioassay

ADH is extracted from plasma and assayed in water-loaded rats by measuring the change in urine conductivity.

Fluid: Plasma
Volume: 10 ml (estimated)
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated plasma at -20°C. The effect of this type of storage upon ADH concentrations would have to be determined by laboratory study.

Problems: Anticoagulants would be required. See Preservation. The volume of plasma required may not need to be so large. A laboratory study might develop more sensitive techniques requiring only 1 ml of plasma or less.

References: 104.

29. Adrenocorticotrophic Hormone (ACTH): Method: Bioassay Technique

Corticosterone release from the adrenals in rats whose pituitary secretion has been halted by hypophysectomy or treatment with Dexamethasone, is produced by ACTH in the plasma from a test subject. The amount of corticosterone released by the rat adrenals is propoertional to the concentration of ACTH in test plasma. Corticosterone is measured by fluorimetric assay.

Fluid: Plasma
Volume: 2 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated plasma at -20°C. The effects of this type of preservation upon ACTH levels would have to be standardized by laboratory study.
Problems: Anticoagulants would be required.

References: 109-112.

30. Hematocrit: Method: Microhematocrit Technique

Whole blood is drawn into heparinized capillary tubes. One end is sealed. The tubes are then centrifuged, and the fraction of fluid length occupied by packed red cells is measured.

Fluid: Whole Blood
Volume: 60-100 microliters.
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: No method of preservation can be used to obtain accurate and reliable hematocrits on stored blood. This test must, of necessity, be done on board if at all. Elaborate low-temperature freezing techniques utilizing protective adjuvants are necessary to recover a large fraction of erythrocytes. But this fraction is unreliable: it varies too widely from test to test. Because of its relative simplicity and importance to clinical hematological diagnosis, a method of on-board determination employing a centrifuge should certainly be considered.

Problems: See Preservation.

References: 1, 2, 3, 9-23.

31. Reticulocyte Count: Method: Counting of Stained Blood Smear

Blood smears are stained with methylene blue or cresyl blue, and the fraction of reticulocytes among red cells is determined by counting.

Fluid: Whole Blood
Volume: 0.1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Little information exists concerning the effects of storage of whole blood upon reticulocyte counts, but considering the difficulty in storing the formed elements of blood, it does not seem feasible to do reticulocyte counts on blood stored in any manner. Since the usual method of counting makes use of blood smears, it might be possible to fix and store air-dried smears made during space flight for later analysis. This would require laboratory study, however, to develop an ideal, standard technique. Safety hazards in the use of a fixative would have to be assessed and adequate controls developed. See drawings.
Problems: See Preservation.

References: 1, 9-23.

32. RBC (Total): Method: Counting in Hemocytometer

The usual method employed on fresh blood is to dilute quantitatively a known volume of blood and count the erythrocytes in the wells of a hemocytometer which contains carefully ruled gratings and a known chamber volume for this procedure. Automatic counting (Coulter Counter) can also be done.

Fluid: Whole Blood
Volume: 0.1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: There is no way of preserving whole blood for later absolute, accurate cell counting. Elaborate low-temperature freezing techniques exist which would be too complicated and uneconomical in terms of power and weight penalties to employ on spacecraft. Even with the best technique, there is some loss of blood cells and results are not always reproducible. For these reasons, it might be

worthwhile to resort to a chemical technique of preservation using formalin (58). This technique is not standard and would have to be simplified for use in space. The safety hazards involved would have to be assessed and adequate controls developed. A laboratory developmental study would be required.
Problems: See Preservation.

References: 1, 9-23, 24.

33. WBC (Total): Method: Counting in Hemocytometer

Counts are made as for erythrocytes except that the diluting fluid used is acetic or hydrochloric acid which hemolyzes red cells, leaving the white cells intact.

Fluid: Whole Blood
Volume: 0.1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: See RBC (Total). The formalin preservation technique could probably be modified to preserve leukocytes alone by hemolyzing red cells prior to adding preservative. As for erthrocytes, this method would require a laboratory study for development. Techniques for preserving leukocytes by freezing are even more elaborate and unreliable than those for erythrocytes.
Problems: See Preservation.

References: 1, 24, 25-28.

34. WBC Differential: Method: Differential counting of stained, air-dried blood smear

A blood smear on glass slide is air-dried, stained with Wright's or Giemsa stain and the proportion of the various types of leukocytes is determined by counting. (See Figure 3-1.)

Fluid: Whole Blood
Volume: 0.1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: It may be possible to store air-dried blood films in some chemical preservative such as methanol, ethanol, or formalin for postflight staining and counting. Whether or not this or some other approach is feasible would have to be determined by a laboratory study, hopefully leading to definitive techniques. Safety hazards involved in using such fixatives would have to be assessed and adequate controls developed.

PREPARATION OF BLOOD SMEARS

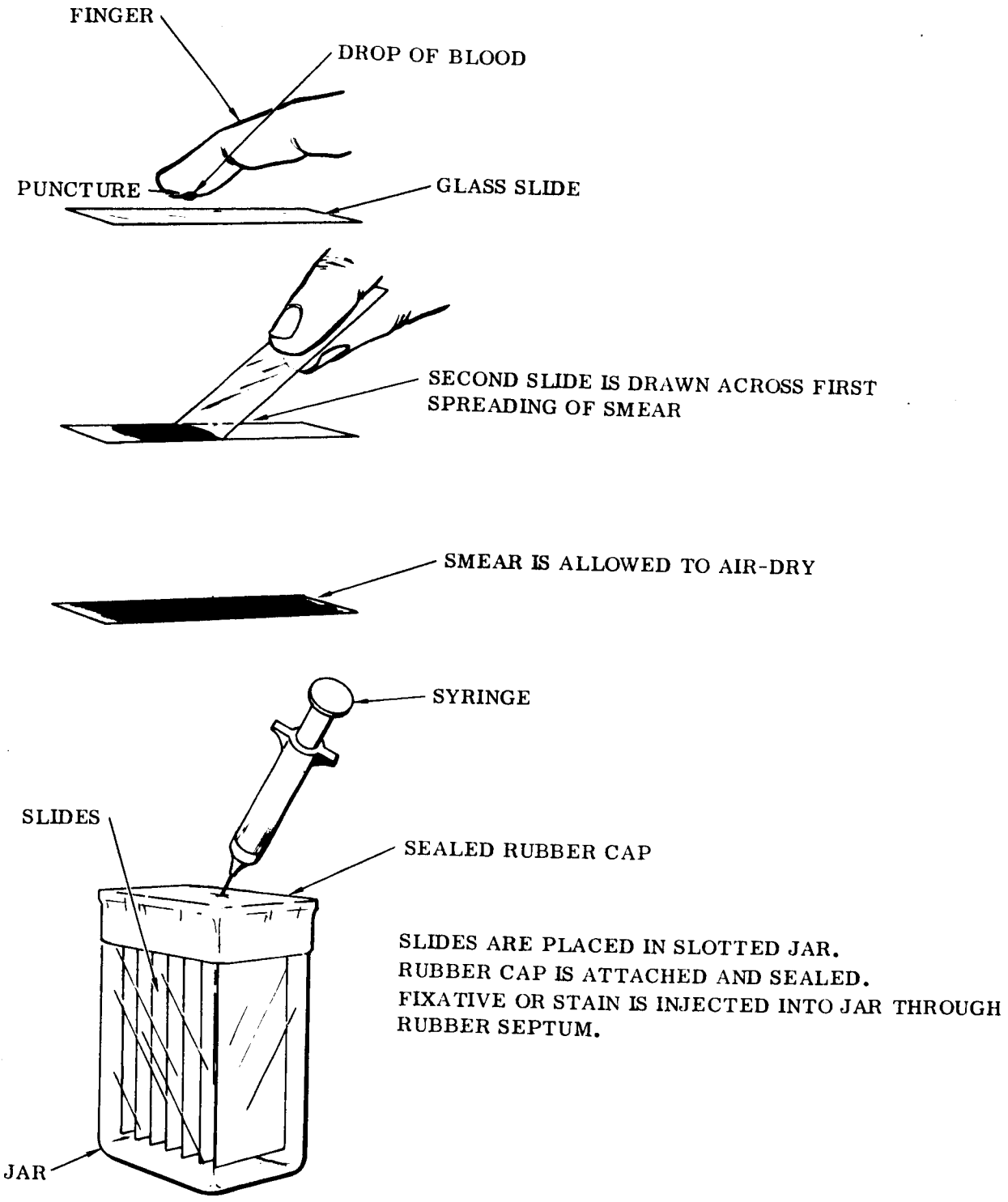

Figure 3-1. Preparation of Blood Smears

Problems: See Preservation.

References: 1, 9-23, 25-28.

35. RBC Cell Mass (Isotopes): Method: Isotopic labeling of blood components

Red cell mass may be determined by RISA^{125} labeling of plasma. After injecting a known amount of RISA^{125}, a sample of circulating blood is withdrawn and a hematocrit is measured at the same time. After calculating the isotopic dilution, plasma volume and red cell mass can be determined. Another technique employs simultaneous labeling of plasma (RISA^{125}) and red cells (Cr^{51}) and differential counting of samples by spectrometric analysis of their characteristic gamma emissions.

Fluid: Whole Blood
Volume: 1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Radioactivity is not affected by the method of storage, and preservation of intact cells is not necessary if hematocrit is determined at time of sampling. Refrigeration or freezing would be necessary to prevent degradation and to preserve original volume.
Problems: The use of a single injection of isotopes over a long period of time would have to be quantitated for the tests in mind. The alternative of injecting isotopes in flight would not be recommended unless a physician were on board. Laboratory study would be necessary to modify existing techniques. In-flight hematocrit determination at time of sampling would be necessary.

References: 1, 29.

(Hematocrit is defined as the volume percent of erythrocytes (also called cell volume percent) in blood and is a relative measure obtained from a small sample of total blood in the body. It is obtained by centrifuging an aliquote of blood and measuring the fraction of tube length originally filled with blood, which is occupied by packed red cells. For example, tube length filled with fluid is 10.0 cm. Length occupied by red cells is the bottom 4.0 cm. Hematocrit is thus 40 vol. percent.

Red cell mass, on the other hand, is an absolute measure of the total volume of erythrocytes in the blood of the body. For example, blood volume may be 5000 ml. Red cell mass might be 2000 ml. It is usually measured by isotopic dilution techniques which yield a measure of blood volume. If the hematocrit is known, red cell mass can be determined from this.)

36. RBC Survival: Method: Isotopic labeling of red cells

An aliquot of subject's blood is withdrawn and the red cells are labeled with Cr^{51}. The blood is then reinjected, and the loss of radiochromium from the circulation after correction for radioactive decay is a measure of red cell survival.

Fluid: Whole Blood
Volume: 1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Same comments as for RBC Cell Mass. Samples are withdrawn over a period of time to determine survival rate.
Problems: Same as RBC Cell Mass.

References: 1, 29.

37. Hemoglobin: Method: Oxyhemoglobin Method (Macro); Cyanmethemoglobin Method (Micro and Ultramicro)

Oxyhemoglobin stabilized in dilute ammonium hydroxide solution is measured photometrically at 540 millimicrons (macro method). Cyanmethemoglobin is produced from all forms of hemoglobin in the blood by a reaction whose first step is conversion with ferricyanide to methemoglobin which then reacts with cyanide to form cyanmethemoglobin. This is measured photometrically at 540 millimicrons (micro) or 545 millimicrons (ultramicro). (See Figure 3-2.)

Fluid: Whole Blood
Volume: 0.1 ml Macro
20 micro l. Micro
1.5 micro l. Ultramicro

	MACRO	MICRO	ULTRAMICRO
Sensitivity:			
Precision:	± 3%	± 3%	± 3%

Accuracy: Abnormal hemoglobins in both methods increase values.
Preservation: Freezing and storage of a dilute, hemolyzed sample at -20°C.
Problems: The withdrawal of a known volume of blood and the addition of diluent must be done during flight. The use of non-wettable micropipettes in the form of syringes with blood obtained by skin puncture would probably be the best method of withdrawal. These pipettes would then be fitted with needles, and the blood would be injected through rubber septa into bottles containing the proper volume of diluent. The choice of hemolyzing diluent and the engineering problems involved would require a laboratory study which would result in the design of appropriate hardware. See Section on Collection.

References: 1, 2, 53.

PREPARATION OF HEMOLYZED BLOOD SAMPLES FOR
HEMOGLOBIN AND METHEMOGLOBIN

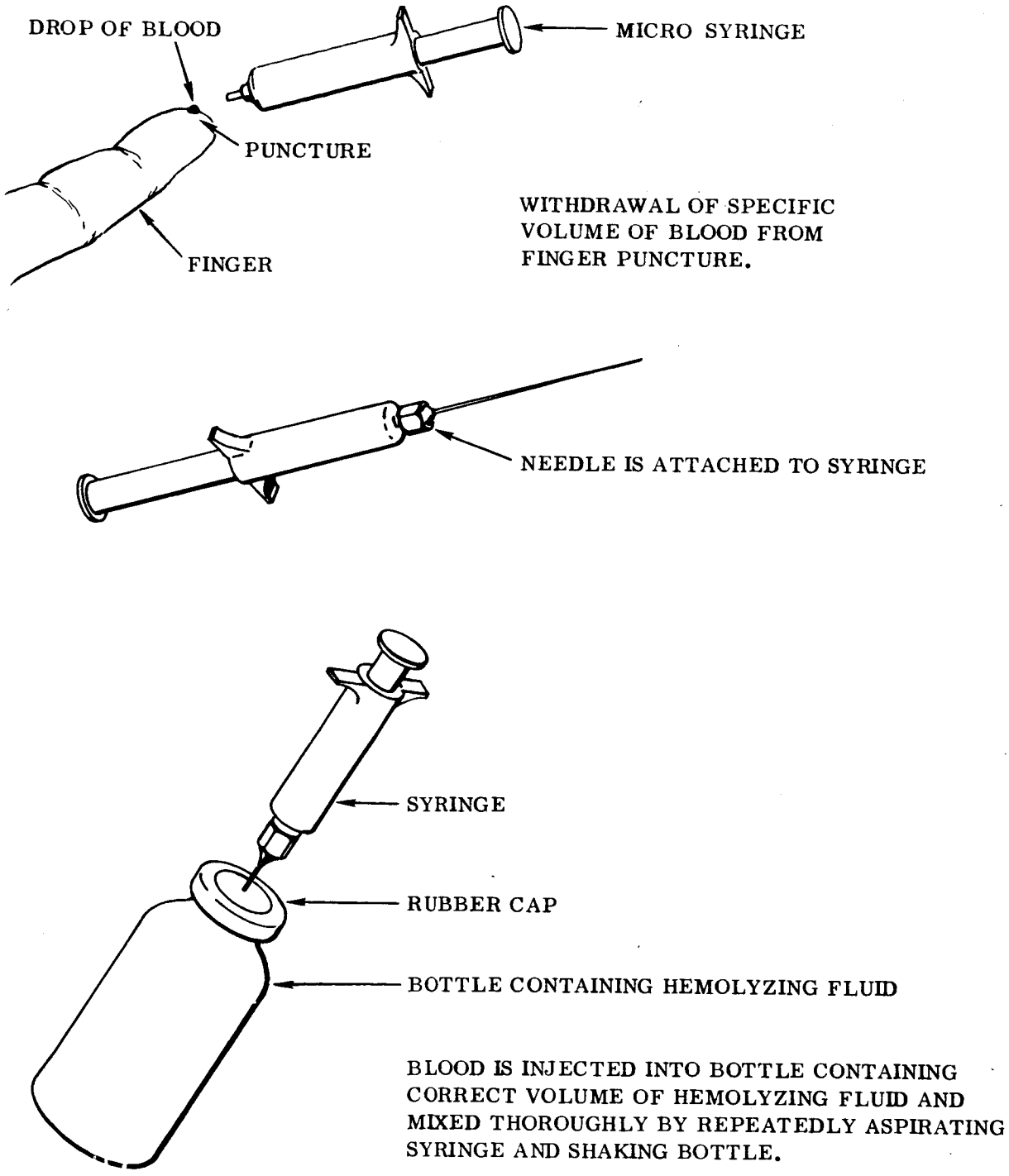

Figure 3-2. Preparation of Hemolyzed Blood Samples

38. Platelet Count (Estimate): Method: Indirect counting method of Dameshek

Platelets are counted under the microscope in a blood smear stained with cresyl blue.

Fluid: Whole Blood
Volume: 0.1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy: Low level of accuracy, but alternatives not applicable to space sampling and storage problems.
Preservation: There is no known method of preserving platelets unaltered. For this reason, it might be best to resort to a chemical technique of preserving air-dried blood smears for later staining and counting. The actual techniques and the reliability of using such a procedure for platelet estimates would have to be investigated in a laboratory study.
Problems: See Preservation.

References: 1, 9, 20, 30-34, 96.

39. Plasma Volume: Method: Isotopic dilution technique employing radioiodinated serum albumin ($RISA^{125}$)

An aliquot of $RISA^{125}$ of known total radioactivity is injected into subject. After mixing, a blood sample is withdrawn, and the serum radioactivity is measured as well as hematocrit. Total Plasma Volume can then be determined.

Fluid: Serum
Volume: 1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Separated serum can be stored in any way for this test in closed containers (necessary because volume of sample must later be accurately determined). Refrigeration or freezing is recommended to prevent degradation.
Problems: Requires in-flight determination of hematocrit at time of sampling. Although the usual method employs a blood sample of 10 ml, the sample size could be reduced by using I^{125} in greater quantity or of a higher specific activity, or use of more sensitive measuring equipment.

References: 1, 113, 114.

40. WBC Motility and Phagocytic Activity: Method: Microscopic Observation of of Leukocyte Movement and Phagocytosis

White cell movement is observed with a microscope. They are exposed to particulates such as iron filings or latex particles, and the degree of phagocytosis is observed.

Fluid: Whole Blood
Volume: 20 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Only the most elaborate, low temperature, quick-freeze techniques using protective adjuvants will preserve motility and phagocytic activity to any degree whatever. Even then, recovery is small and unreliable. Contact with citrate anticoagulants destroys all signs of phagocytosis very quickly. Because of the difficulties involved, an in-flight alternative would have to be devised by a laboratory study leading to hardware design, or the test should be omitted.
Problems: See Preservation. Heparin is required as an anticoagulant.

References: 1, 20, 25-28.

41. Platelet Adhesiveness: Comparative Counting

Absolute platelet counts are determined from two samples of blood: one is withdrawn directly from a vein in one arm; the other is withdrawn from a vein in the other arm through a column of tiny glass beads to which some platelets adhere. The difference in the two platelet counts thus obtained is a measure of platelet adhesiveness.

Fluid: Whole Blood
Volume: 16 ml total (8 ml from each bleeding site)
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Formed elements of blood are extremely difficult to preserve. A laboratory study would be required to determine the feasibility of performing this test on preserved samples.
Problems: The technique of semi-quantitative estimation of platelet adhesiveness is restricted to advanced laboratories with specially trained personnel and the results are open to interpretation. For other problems, see preservation.

References: 6, 106-108.

42. Fibrinogen: Method: Fibrinogen Test - Andersch-Gibson Modification

Fibrin obtained from clotted plasma is hydrolyzed, and the resulting tyrosine forms a colored product when reacted with a phenol reagent. The density is compared spectrophotometrically with a standard tyrosine solution, and the amount of fibrinogen is calculated.

Fluid: Plasma
Volume: 1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated plasma at -20°C. Information is not available concerning the effect of this type of storage on fibrinogen concentration of plasma, but from other data there is reason to believe that little change will occur.
Problems: Oxalate anticoagulants must be used to prevent clotting. Combining blood and clotting agents in zero gravity presents certain problems which are discussed elsewhere.

References: 1, 35, 97.

43. Fibrinolytic Activity: Method: Stefanini-Dameshek Screening Test

Subject plasma and control plasma are compared for the length of time it takes a fibrin clot to lyse.

Fluid: Plasma
Volume: 1.5 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated plasma at -20°C. Information is unavailable concerning the effect of storage on fibrinolytic activity. A laboratory study would be necessary to determine this.
Problem: Citrated plasma is required for this test.

References: 1.

44. Prothrombin Activity: Method: Specific Determination of Prothrombin-Owren

Clotting time of known reaction mixture of clotting agents plus test subject's prothrombin is determined. Specific test for prothrombin activity alone.

Fluid: Plasma
Volume: 0.2 ml maximum
Sensitivity:)
Accuracy:) not a quantitative test
Precision:)
Preservation: Freezing and storage of separated plasma at -20°C. Some data exists which shows that prothrombin activity may be changed by this method of storage. In view of this, a laboratory study would be required to standardize the extent of change over the period which might be used for a space experiment.
Problems: Oxalated plasma is required for this test.

References: 1, 36, 98.

45. Plasma Thromboplastic Component (PTC): Method: Assay for Blood Clotting Factor IX

Clotting times of diluted mixtures of test subject's plasma and PTC deficient plasma are determined.

Fluid: Plasma
Volume: 1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated plasma at -20°C. Little change in PTC is expected using this method of storage, but this would have to be established by a laboratory investigation.
Problems: Oxalate anticoagulants must be used to prevent plasma from clotting.

References: 1, 37, 38, 97.

46. Antihemophilic Globulin (AHG): Method of Jung, modified

All necessary coagulation factors of the test system are kept constant except for AHG. Clotting time is measured and is thus dependent only on the level of AHG in test plasma.

Fluid: Plasma
Volume: 0.1 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated plasma at -20°C. There are a number of reports which indicate that AHG is unstable when preserved in this way. This would have to be established by a laboratory study; standard curves of the changes could be plotted from a number of determinations.
Problems: Citrated plasma is required. Platelet-poor plasma is required for storage which necessitates high-speed centrifugation. AHG is unstable in the presence of even trace amounts of thrombin which means that blood must be thoroughly mixed with anticoagulants and no trace of clot formation in the container is permissible.

References: 1, 37, 39-43, 97.

47. Immunoglobulins: Method: Paper Electrophoresis

For a description of paper electrophoresis, see serum proteins. Immunoglobulins are defined only by their electrophoretic pattern and other physical characteristics. The immunoglobulins are included in the β globulin peak and are not detectable when small amounts of serum are used. With larger amounts of serum, separate peaks have been noted, but neither the physiological role nor the clinical significance of the immunoglobulins are known. Determination of immunoglobulins is still in the experimental stage.

Fluid: Serum
Volume: 5-10 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)
Preservation: Freezing and storage of separated serum at -20°C. The effect of this type of storage upon immunoglobulins would have to be determined by a laboratory study.
Problems: See preservation.

References: 105.

48. Karyotyping: Method: Phytohemagglutinin Incubation - Arakaki

Whole blood or plasma containing leukocytes is incubated with phytohemagglutinin for three days at 37°C in a special tissue culture medium. Colchicine is added to the cultures a few hours before harvesting, and the leukocytes are spread on glass slides, fixed and stained with aceto-orcein or Feulgen reagent. Dividing metaphase cells are photographed, and the morphology of their chromosomes is analyzed.

Fluid: Whole Blood
Volume: 0.1 - 0.2 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy:)

Preservation: Techniques for preserving viable leukocytes for long periods of time are extremely complicated and uneconomical, involving elaborate separation methods, and cryogenic freezing and storage. Whether or not moderate freezing and storage at -20°C could be employed would have to be studied. If this does not prove feasible, on-board culturing of leukocytes might be possible but would require the development of special techniques and hardware in order to deal with the absence of gravity and unskilled personnel.
Problems: See Preservation

References: 25-28, 44-48

49. Clotting Time: Method: Capillary Tube Method

Blood is obtained from skin puncture and is allowed to enter several 3-4 inch capillary tubes (un-heparinized). Small pieces of tubes are broken off at 30 second intervals until a fine thread of fibrin persists at site of break. The time from skin puncture to the formation of fibrin thread is the clotting time.

Fluid: Whole Blood
Volume: 0.1 - 0.5 ml
Sensitivity:)
Precision:) not a quantitative test
Accuracy: See Problems)
Preservation: Clotting time cannot be determined upon stored blood. It must be measured at the time of withdrawl. The above-described technique was chosen because it could easily be adapted to on-board determinations in zero gravity.
Problems: The method described is inexact and usually is not employed where accuracy is desired. However, it is the only technique available which is easily adaptable to zero gravity. Contamination with tissue fluid is the chief source of error. Fragments of glass produced by capillary fracture might produce a hazard in space cabins, but this could be prevented by breaking the tubes in flexible, tough, transparent plastic bags (Figure 3-3).

References: 1, 6, 37, 39.

50. Clot Retraction: Method: Observation of Clot Retraction

Blood is placed in a clean test tube in a 37°C water bath and is inspected for the time that retraction begins and the time it ends. This usually takes 24 hours. The quality of the clot is observed, and the amount of serum extruded can be measured.

Fluid: Whole Blood
Volume: 2 - 3 ml
Sensitivity:)
Precision:) Not a quantitative test.
Accuracy:)
Preservation: Clot retraction cannot be measured on stored blood. A method would thus have to be devised to do this study during flight, if at all. It might be possible to employ an on-board centrifuge for this purpose, rotating at a speed such that one-G would be generated. But this would mean tying up the centrifuge for 24 hours for any one set of determinations. Since this parameter of blood would not be expected to change rapidly, it might be possible to study it once a week or less frequently. Closed tubes containing blood could be inspected for clot retraction either by a stroboscopic technique or by stopping the centrifuge and carefully examining the tubes.
Problems: See preservation.

References: 1, 6, 37, 39, 49.

51. LDH Isozymes: Method: Starch gel electrophoresis and enzyme activity tests

Lactic acid dehydrogenase isoenzymes move at different rates in electric fields with certain buffers. The amount of each isoenzyme thus separated is then determined by measuring the rate of substrate utilization spectrophotometrically.

Fluid: Serum
Volume: 0.1 ml
Sensitivity:
Precision: $\pm$ 6%
Accuracy: Difficult to assess.
Preservation: Freezing and storage of isolated serum at -20°C. Available data shows that this method of preservation will produce changes in various isoenzyme activities to different extents. A laboratory study would be necessary to standardize these changes.
Problems: See Preservation. Hemolysis must be avoided because red cells contain higher concentrations of LDH isozymes.

References: 6, 50-53.

52. Immune Bodies: Methods: Immunoelectrophoresis
Agglutination
Precipitin Test
Complement Fixation Test

Immunoelectrophoresis: This technique combines immunological tests for specific antibodies with electrophoresis.

Agglutination - antigen (cellular or particulate) is in suspension. Antigen agglutinates (clumps) and settles out after it reacts with its specific antibody.

Fluid: Serum
Volume: 0.2 ml per antigen tested.

Precipitin Test - a soluble antigen (in solution) reacts with its antibody producing precipitation and settling out of the antigen.

Fluid: Serum
Volume: 0.1 ml per antigen tested

Complement Fixation Test - use is made of exogenous complement (from guinea pig blood) and sheep erythrocytes as sensitive indicators of antigen-antibody reactions. Complement becomes bound or fixed in antigen-antibody reactions. If hemolysis occurs when sensitized sheep erythrocytes are added to the system, free complement is present, and the unknown serum contains no antibody. If hemolysis does not develop, complement has been bound by the antigen-antibody complex, and the unknown serum is considered positive. For this test, the unknown sera must be heated at 56^{o}C for 30 minutes to destroy endogenous complement.

Fluid: Serum
Volume: 0.6 ml per antigen tested

Preservation: Freezing and storage of separated serum at -20^{o}C. This type of preservation can be used for an indefinite period of time. Chemical preservation using merthiolate combined with refrigeration can also be used, but length of storage permissible is unknown. Since freezing is the method of choice for most analyses, it would be uneconomical to store samples for the study of immune bodies in a separate fashion especially if only a fraction of a given serum sample will be used for these tests.
Problems: Heat treatment of serum for complement fixation test can be carried out after samples are returned to laboratory. The only other problem is that the total volume of serum necessary is undefined since this depends on the number of antibody titers to be determined. Volumes noted are for a single antibody - antigen test.

References: 1.

53. Transferrins: Method: Determination of Iron-Binding Capacity

Excess iron is added to serum as ferric ammonium citrate to saturate the transferrins. Excess iron is removed with ion exchange resin, and the bound iron remaining in the supernate is the iron-binding capacity. The iron is determined by freeing it from protein with hot trichloroacetic acid, reducing it with hydrazine and measuring it spectrophotometrically by reaction with sulfonated orthophenanthroline.

Fluid: Serum
Volume: 0.5 - 1.0 ml
Sensitivity:
Precision: $\pm$ 5%
Accuracy:
Preservation: Freezing and storage of separated serum at -20°C. Stability of transferrins under these conditions is believed to be good, but a laboratory study would be necessary to establish changes, if any.
Problem: Trace contamination with iron can seriously alter results; scrupulously clean equipment required. Hemolysis must be avoided.

References: 53.

54. Methemoglobin: Method: Evelyn and Malloy, modified

Methemoglobin has a chracteristic absorption band at 635 millimicrons which is completely abolished by the addition of cyanide. The change in optical density is proportional to methemoglobin concentration.

Fluid: Whole Blood
Volume: 0.1 ml
Sensitivity:
Precision: $\pm$ 5%
Accuracy:
Preservation: Freezing and storage of a hemolyzed sample of blood at -20°C. This technique is suggested although it is not a standard procedure. A laboratory study would be necessary to develop and standardize a sampling and storage procedure for use in space flight. See Hemoglobin.
Problems: Any deterioration of blood may change the value of methemoglobin concentration. Hemolyzing solution volumes required, determination of volume sampled would all require study. See Hemoglobin.

References: 2, 6, 53, 99.

CAPILLARY TUBE METHOD OF DETERMINING CLOTTING TIME

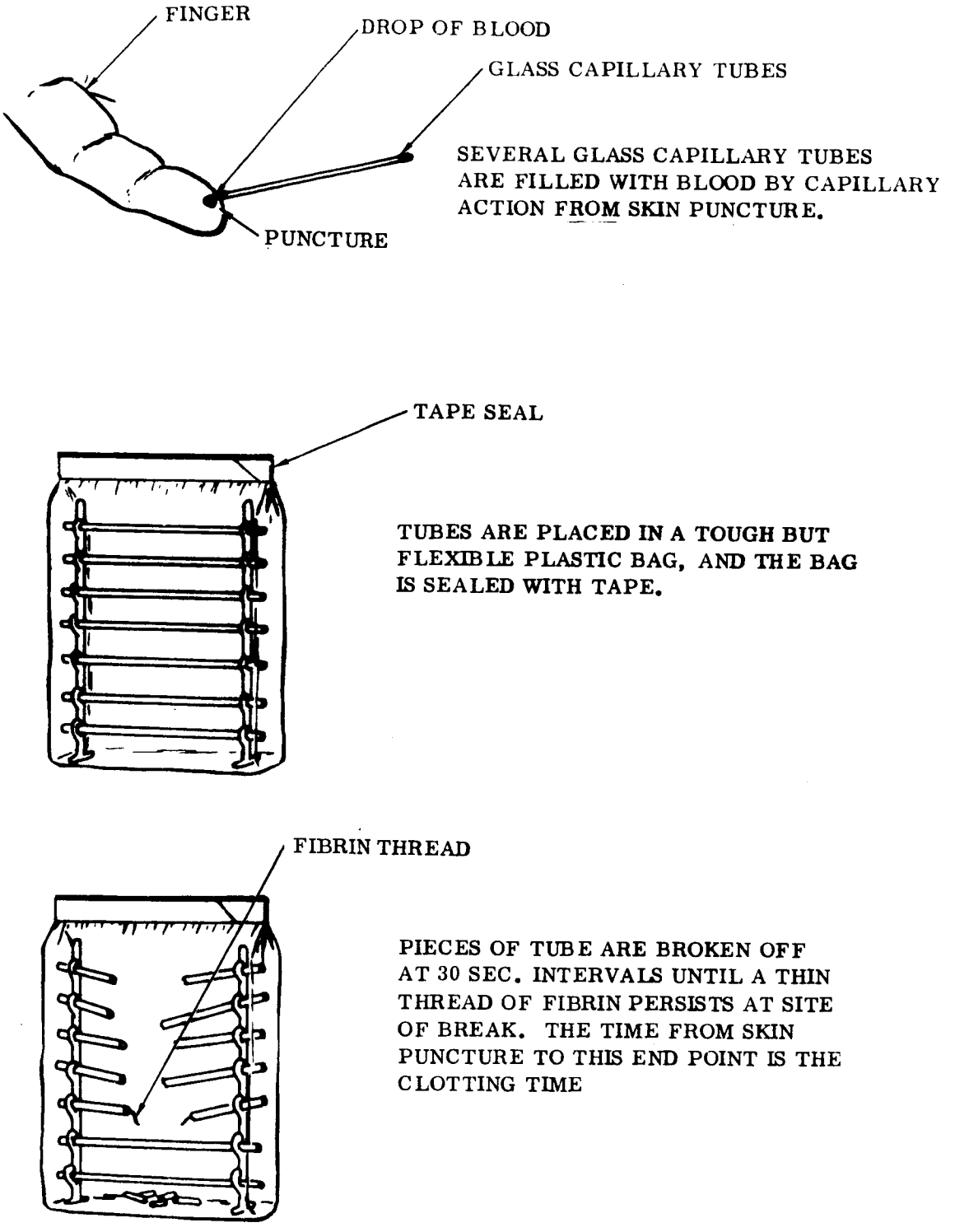

Figure 3-3. Capillary Tube Method of Determining Clotting Time

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3.2 URINE

Approximately 450 references pertaining to urine were obtained by a search of the literature. About ninety of these were directly applicable to this study and are listed as part of the bibliography.

Various other publications in leaflet or pamphlet form were used and are included in the bibliography.

Before selecting the "best" procedures for analyzing urine constituents, some thought was devoted to the question of "best for what?" Obviously, one procedure is best in terms of time required for the assay, another in terms of convenience, and still another in terms of accuracy. Other procedures can be classified as best in terms of cost, reproducibility, and sensitivity. Since it is impossible to find a procedure that is best with respect to all of these parameters, the question of where to place importance in defining a best method had to be answered.

The major consideration in an analytical procedure is its potential for giving a true value for a selected urine constituent; that is, time requirements, convenience, and cost of the procedure are not as important. Parameters such as accuracy, precision, and specificity, all of which contribute to quality results, are of prime importance in method selection.

The following points summarize the rationale used in selecting best methods for the analysis of urine constituents:

a. The sensitivity of a procedure is related to urine volume requirements. Volume-weight restrictions during space missions limit the amount of urine that can be stored. In general, it is preferred that stored urine sample volumes be as small as possible; in this respect, analytical procedures with the greatest sensitivity were favored as the method of choice.

b. Analytical procedures having the lowest coefficient of variation were selected except when volume requirements were excessive. One aldosterone procedure had an acceptable precision (± 5%) but required 600 ml of urine for the assay. In this case, a procedure was selected with a more compatible urine requirement at the expense of precision. Fortunately, many of the recently developed analytical assays are both sensitive and precise. Many of the selected procedures have precisions of ± 1%, some vary between ± 1% and ± 5%, and a few others as high as ± 10%. However, it should be remembered that these values are very dependent upon the skill of the analyst, and the condition and type of equipment used.

c. The specificity of a procedure contributes significantly to the accuracy of determination. Analytical methods with excellent specificity were selected, provided sensitivity and precision were also good. In some cases, specificity is built into a procedure by modifying it in order to eliminate interferences. This is true of several modifications of the Jaffe reaction for creatinine where interfering chromogens are eliminated by chromatographic techniques. In the double isotope assy for aldosterone, accuracy is attained by correcting for procedural losses. This is accomplished by placing a small amount of tritium-labeled aldosterone in the sample and measuring the amount recovered at the end of the assay. Other determinations are made specific by immunochemical or enzymatic reactions.

d. Numerous clinically accepted methods can be rejected because of the semiquantitative or qualitative nature of the determination. Urine technology literature reveals an abundance of chemical procedures for detecting and quantitating clinically important urine constituents. In many instances, one is hard-pressed to choose among the different methods available for a single constituent, not to mention the large number of modifications that usually accompany the basic procedure. Quantitation is usually obtained through gravimetric, titrimetric, colorimetric, or turbidimetric techniques. Accuracy is lost through interferences from other urine constituents or urine preservatives. Normally, many of the routine chemical methods can be rejected on urine-volume requirements alone (sensitivity).

1. Creatine, Fluorimetric Assay: The ninhydrin reaction of Conn.

Creatine is isolated chromatographically, subsequently eluted, and coupled with ninhydrin reagent. The adduct fluoresces and is quantitated via this property. Creatinine does not couple with ninhydrin under the conditions used.

Sensitivity: 0.7 μg/ml.

Urine Required: 1 ml, and recoveries are essentially quantitative.

References: 1, 37, 40, 60, 72, 78, 99, 100, 117-9.

2. Creatinine: Folin Colorimetric Analysis

Creatinine is isolated on Lloyd's reagent, subsequently eluted, and subjected to a non-hydrolytic Jaffe reaction with picric acid. Proteins are first precipitated by trichloracetic acid. The pH of the deproteinization reaction should be less than two to prevent creatinine loss by adsorption on the precipitated protein. Recoveries are quantitative under these conditions.

Precision: ±5%

Urine Required: 0.1 ml to be diluted.

References: 1, 40, 60, 113, 117, 118

Note. Creatine is often determined by the Jaffee reaction too, but some doubt exists as to the complete conversion of creatine to creatinine. Thus, creatine is determined to be more nearly accurate by the ninhydrin reaction of Conn.

3. Chloride: Potentiometric Assay

Chloride ion concentration is determined by measuring the potential difference generated when silver is converted to silver ions. The potential developed is a function of chloride ion concentration. Silver chloride coated silver electrodes are employed and the millivolt potential is read from the dial of a pH meter. A millivolt vs. chloride ion concentrations plot obtained from standard solutions is used to convert the millivolt value of the unknown solution to units of concentration.

Precision: ±2%

Urine Required: 0.5 to 1.0 ml.

References: 1, 43, 46, 94, 109, 118.

4. Inorganic Sulfate: Spectrophotometric Assay. The modified method of Kleeman, Taborsky, and Epstein.

Protein and phosphate are first precipitated simultaneously with uranyl acetate. Sulfate is then precipitated with benzidine. The benzidine is then released from the precipitate and coupled with 2-naphthoquinone-4-sulfonate to produce a colored product which is quantitated.

Precision: ± 5%

Urine Required: 0.1 ml to be diluted.

References: 1, 36, 102, 103, 118, 120, 123.

5&6. Sodium and Potassium: Flame Photometry

Quantitation by emission spectrophotometry.
Accurate and specific.

Precision: ±0.5 to ± 2.5%.

Urine Required: 0.1 to 0.5 ml.

References: 1, 3, 39, 46, 98, 118.

7. Calcium: Atomic Absorption Spectrophotometry

Highly specific and accurate.

Precision: ± 1%.

Urine Required: 0.1 to 1.0 ml.

References: 1, 2, 3-7, 9, 10, 13, 47, 51, 61, 62, 63, 101, 115, 118.

8. Magnesium: Atomic Absorption Spectrophotometry

Highly specific and accurate.

Precision: ± 1.0%.

Urine Required: 0.1 to 1.0 ml.

References: 1, 2, 10, 13, 48, 51, 57, 58, 62, 63, 89, 92, 96, 118.

9. Zinc: Atomic Absorption Spectrophotometry

Precision: ± 1%.

Urine Required: 0.1 to 1.0 ml

Sensitivity: 0.002 mg/ml

References: 13, 42, 49, 51, 68, 91, 118.

10. Managanese: Atomic Absorption Spectrophotometry

Precision: ± 1%

Urine Required: 0.1 to 1.0 ml

References: 10, 14, 15, 66, 73, 118.

11. Proteins: Kjeldahl Assay

Semi-micro Kjeldahl procedure using a mercury (some investigators prefer Selenium) catalyst. Ammonia absorbed in 4% boric acid solution. The procedure is applied to the washed tungstic acid precipitate of the urine sample. Convert N_2 to protein. Accuracy is only fair due to the factor 6.25.

Precision: ±1%

Urine Required: 0.2 to 1.0 ml

References: 1, 11, 16, 83, 95, 118.

12. Non-Protein Nitrogen: Kjeldahl Assay

Semi-micro Kjeldahl procedure using a mercury catalyst. Ammonia absorbed in 4% boric acid solution. The procedure is applied to the filtrate or supernatant plus washings of the protein determination (#11).

Precision: ± 1%

Urine Required: 0.2 to 1.0 ml

References: 1, 16.

13. Total Nitrogen: Kjeldahl Assay

Nitrogen of #11 plus nitrogen of #12.

Precision: ± 1%

Urine Required: 0.2 to 1.0 ml

References: 1, 16.

14. 17-Hydroxycorticosteroids. Spectrophotometric

Via Few's Modification of the Norymberski Method. Involves reduction with sodium borohydride followed by oxidation with sodium periodate. Derivatives are complexed with alkaline m-din, trobenzene and quantitated colorimetrically.

Precision: ± 4% to ±9%

Urine Required: 5.0 ml

Accuracy: 90% to 99%

References: 8, 16, 19, 41, 45, 50, 53, 55, 75-7, 86, 90, 97, 116.

15. Aldosterone: Double Isotope Assay

Tritium labelled aldosterone is added to the urine sample. Total steroids are then isolated by thin layers chromatography and acetylated with C14 acetic anhydride. The doubly labelled aldosterone acetate is then isolated vy a combination of thin layer adsorption and paper chromatography. Correction for losses is based upon the diminution in tritium lable and quantitation is effected by the carbon 14 label.

Precision: ± 10%

Urine Required: 15 ml.

References: 12, 16, 33, 81, 87, 118, 121, 122.

16. Vasopressin - Bioassay: Hydrated Rat Assay

Brattleboro strain rats with hereditary hypothalamic diabetes insipidus having no endogenous synthesis of vasopressin are used as test animals. This condition provides for a stable baseline diuresis needed before injections of test substances. Normal rats can also be used but stable baseline diuresis is established by ethanol intoxication via stomach tube or I. V. injection. Test animals are hydrated during the entire assay.

When a constant diuresis has been attained, test substances such as plasma, serum, or urine are injected I. V. and the response from any antidiuretic substance in the test material is measured by change in urine output and concentration. A 0. 2 to 1. 0 ml of serum or plasma are required for assay; the volume of urine required was not found in the literature.

Standard curves are obtained by injections of known amounts of vasopressin. Sensitivity is about ±2 μU (USP) vasopressin.

References: 121, 138, 141 - 144.

17. Serotonin: Acid Fluorimetric Assay. Method of Davis, et al

Serotonin glucuronide and serotonin sulfate are cleaved enzymatically. Free serotonin is then isolated by extraction and ion exchange procedures. Maximum fluorescence is obtained in 3N HCl.

Sensitivity: 0.2 to 0.3 μg.

Specific due to isolation techniques.

Urine Required: 0.1 ml to be diluted.

Precision: ± 6 - ± 10%

References: 67, 117, 124, 125, 11, 82, 118, 132, 133.

18. Mucoproteins: Colorimetric Assay

Mucoprotein is selectively precipitated with ethanol and quantitated by measuring the carbohydrate moiety. Precipitated mucoprotein is dissolved in an aqueous sulfuric acid solution and reacted with resorcinol to produce a colored product. Mannose and galatose standards are used.

Precision: ± 2%

Urine Required: 0.2 ml

No accuracy figures were available.

References: 1, 35, 11, 16, 38, 84, 93.

19. Standard Clinical Analysis:

a. Volume - Urine constituent values are generally expressed on the basis of 24-hour output. Thus, it is important to know the total urine volume during that period. A single specimen volume is often measured directly; the fraction of total daily output is then calculated from a normal average figure (1500 ml/24 hours).

Another method is to collect the entire 24-hour output in a preweighted container. The urine volume is then calculated from weight and specific gravity values.

References: 1, 11, 16.

Neither of these methods is adequate for zero gravity in-flight determination of 24-hour urine volume. A simple, manual method would be to measure the urine at each voiding by compressing the liquid in a graduated container to remove all air.

Two systems which have been developed by the General Electric Company for semi-automatic and automatic collection of urine samples are described in Section 6.1.2.

b. pH - Urine pH values are accurately determined with a pH meter. Clinically, this accuracy is not required and pH indicator paper is used to make the determination. Paper with a sensitivity of 0.1 pH unit is available, but a sensitivity of 0.5 pH unit would seem adequate. Nitrzine paper covering the pH range of 4.5-7.5 is widely used as is wide range Hydrion paper. Urine pH varies, in a healthy adult between 4.6 and 8.0.

References: 1, 16, 71.

It is suggested that urine pH values be obtained during collection procedures in-flight. pH indicator paper with a sensitivity of 0.5 pH unit is adequate. The pH of preserved urine would be unreliable in reflecting the original value due to:

1. CO_2 and NH_3 losses through lyophilization.

2. Changes induced by many chemical preservatives.

3. Precipitation and sedimentation due to freezing.

c. GLUCOSE: Enzymatic-photometric Method

Glucose oxidase-peroxidase system oxidizes glucose to gluconic acid. Formed H_2O_2 is cleaved by peroxidase and the oxygen liberated oxidizes o-tolidine to form a colored product which is measured spectrophotometrically. Inhibitors of the

system (uric acid and ascorbic acid) are first removed from urine by adsorption. Manual and automated techniques are in use.

Highly specific.
Urine Required: 20 lambda.
Results are reproducible.
Precision: ± 2%.
References: 1, 11, 12, 44, 46, 54, 59, 79, 80, 88, 108, 117, 139.

d. ACETONE BODIES: Fluorimetric Assay

Acetone bodies in urine: acetone: 2%
acetoacetic acid: 20%
β-hydroxybutyric acid: 78%

Most methods measure only acetone and suffer the disadvantages of interference, inaccuracy, and qualitative estimations. The fluorimetric assay measures acetoacetic acid and β-hydroxybutyric acid which make up 98 percent of the ketone bodies in urine.

Conversion of β-hydroxybutyric acid to acetoacetic acid or the reverse is catalyzed by the commercially available enzyme D-β-hydroxybutyric dehydrogenase. Either reaction can be made to go to completion upon proper pH conditions. Depending upon the direction of the reaction, quantitation is obtained by measuring the native fluorescence of $NADH_2$ or the alkaline fluorescence of NAD.

Precision value not available.
Urine Required: 0.2 to 0.5 ml.
Highly specific and accurate.
Precision: ± 2 - ± 5%
References: 1, 34, 56, 74, 117, 118, 126, 134, 135.

e. MICROSCOPY: Quantitative and Morphological Assay

A known urine volume is passed through a membrane filter of known surface area. Formed elements which are retained on the surface of the membrane are fixed, stained, mounted, and examined by light microscopy. Results are expressed as type and number per ml or urine. On the basis of 268 determinations, the following normal values were found:

erythrocytes: 4000 or less/ml
leukocytes: 4000 or less/ml
casts: 30 or less/ml

Procedure can be modified for centrifugation. Formed bodies are resuspended in an isotonic solution which is filtered.

Urine Required: 1.0 ml

References: 1, 11, 16, 52, 64.

f. SPECIFIC GRAVITY: Falling Drop Method

A drop of urine is allowed to fall into a column of an organic solvent mixture, the specific gravity of which is known. Organic mixtures of varying specific gravity are available for urine determinations. If the drop of urine comes to rest after its initial momentum is dissipated, and then neither rises nor falls, the specific gravity of the urine is the same as the solvent mixture. The procedure is more accurate than the hydrometer or "urinometer" method and only requires several drops of urine.

References: 1, 46, 105, 106, 118.

Specific gravity may also be determined by measurement of the refractive index which, in laboratory tests, has proven to be surprisingly accurate (129).

g. ALBUMIN: Immunoassay Procedure

Sensitive and reproducible down to very low concentrations.

Urine Required: less than 0.1 ml.
Precision: $\pm$ 4%

References: 11, 46, 105, 110, 118, 140.

h. TURBIDITY: Nephelometric Determination

Measurement of light scattered at right angles to the incident light beam. The value of nephelometry lies in the ability to measure low concentration of particles which are beyond the sensitivity of turbidimetric measurements. Commercial spectrophotometers for which nephelometric attachments are available are photovolt Lumetron, Model 402E and Coleman Universal Spectrophotometer, Model 14.

References: 1, 11, 117.

Table 3-4 summarizes the information presented on urine constituent analyses.

Table 3-4. Urine Constituent Analyses

Urine Constituent	Analytical Method*	Volume Required (ml)	Precision %	Accuracy**
Creatine	Fluorimetric Assay (3) (ninhydrin reaction of Conn)	0.1	±5	B
Creatinine	Folin Colorimetric Assay (3) (Jaffe reaction)	0.1	±5	B
Chloride	Potentiometric Assay (2)	0.5 - 1.0	±2	A
Sulfate	Spectrophotometric Assay (3)	0.1	±5	B
Sodium	Flame Photometry (2)	0.1 - 0.5	±0.5 - ±2.5	A
Potassium	Flame Photometry (2)	0.1 - 0.5	±0.5 - ±2.5	A
Calcium	Atomic Absorption (2) Spectrophotometry	0.1 - 1.0	±1	A
Magnesium	Atomic Absorption (2) Spectrophotometry	0.1 - 1.0	±1	A
Zinc	Atomic Absorption (2) Spectrophotometry	0.1 - 1.0	±1	A
Manganese	Atomic Absorption (2) Spectrophotometry	0.1 - 1.0	±1	A

*Ref. #1

1 = Macro.
2 = Semi-Micro.
3 = Micro.
4 = Ultra-Micro.

**Ref. #136

A = Highly Specific & Accurate
B = Moderately Specific & Accurate
C = Low Specificity & Accuracy

Table 3-4. Urine Constituent Analyses (Cont)

Urine Constituent	Analytical Method*	Volume Required (ml)	Precision %	Accuracy**
Proteins	Kjeldahl Assay (2)	0.2 - 1.0	±1	C
Total N_2	Kjeldahl Assay (2)	0.2 - 1.0	±1	A
17-hydroxy-corticosteroids	Spectrophotometric (1) (Norymberski Method)	5.0	±4 - ±9	B
Aldosterone	Double Isotope Assay (1)	15	±10	B
Vasopressin	Bioassay Hydrated Rat Assay (2)	0.2 - 1.0 serum or plasma	Reproducible	B
Serotonin	Acid Fluorimetric Assay (3)	0.1	±6 - ±10	A
Mucoproteins	Colorimetric Assay (2)	0.2	±2	C
Glucose	Enzymatic-Photometric Assay (4)	0.02	±2	A
Acetone Bodies	Fluorimetric Assay (2)	0.2 - 0.5	±2 - ±5	A
Albumin	Immuno-Assay (3)	0.1	±4	B
		22.4 - 29.2 TOTAL (45-60 ml for duplicate samples)		

N. B. Elimination of 17-hydroxy-corticosteroids and aldosterone assays from this list would reduce total urine requirements to about 10 ml. Skilled analysts with practiced technique could reduce this requirement to the lower value of about 4 ml.

3.2.1 CORRELATION OF ANALYTICAL METHODS FOR CONSTITUENTS COMMON TO BLOOD AND URINE

Several common constituents are to be measured in more than one biological fluid. In a few cases, different methods are proposed for determination of the same component.

All the methods chosen are satisfactory and would give comparable results, so it is not essential that one standard method be employed in determining each constituent from all sources. Naturally, one method should be decided upon for assaying all samples of one kind of biological material for the same component.

For example, Creatine and Creatinine assays by the direct Jaffe reaction are widely employed in clinical laboratories. Prior adsorption of the creatinine on Lloyd's reagent will reduce the values found by as much as one third, to nearer the "true" amounts present (by elimination of nonspecific chromogens). The ninhydrin reaction for creatine is much more sensitive, of course, but still leaves the creatinine to be determined by one of the other methods.

The conductimetric method for chloride, using a conductimetric titrator, is the method of choice for fecal assays, which may be done directly in this manner. There is no reason why it should not be used on other materials, although the mercurimetric or potentiometric methods are perfectly good alternatives.

In the determination of calcium, Redox titration is more classical and Atomic Absorption Spectrometry more elegant, but the assay with chloranilic acid is widely accepted.

Mucoproteins certainly might as well be tested separately for their tyrosine and protein components in addition to the carbohydrate moiety.

Both the Folin and Wu and notatin methods work well for the measurement of glucose.

Creatine - The tests for blood are modifications of the Folin method which calls for heating or autoclaving samples to convert all creatine to creatinine. The Cohn ninhydrin reaction is used for urine. This requires no preliminary heating. It could also be used for blood, but several texts choose the Folin method for use in standard practice.

Creatinine - Tests for blood and urine are similar.

Mucoproteins - The test for blood calls for analysis of mucoprotein tyrosine, carbohydrate, and protein. The test for urine just analyses the carbohydrate moiety. This may also be sufficient for blood analysis.

Sodium and Potassium - Flame photometry is suggested as the analytical test for both blood and urine.

Chlorides - This test for blood requires a mercurimetric technique while the test for urine calls for potentiometric assay. The latter is a newer technique and is considered quite precise and accurate. The blood test, however, has been accepted as standard by many laboratories for some time and was chosen for this reason. Both tests, however, yield equivalent results.

Calcium - The blood tests employ chemical procedures. These are tests generally used as standard by most clinical laboratories. The test for urine is atomic absorption spectrophotometry which is a newer technique and is considered to be extremely sensitive, precise, and accurate. It has only recently come to be generally accepted; it calls for highly specialized and expensive equipment. This technique could also be used for blood and might have advantages over the chemical techniques described.

Magnesium, Manganese, and Zinc - Both urine and blood tests call for atomic absorption spectrophotometry.

Sulfates - Both urine and blood are analyzed by the same method.

Glucose - Two of the tests for blood employ enzymatic reactions resulting in colored products, as does the test for urine.

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3.3 FECES AND SWEAT

The clinical laboratory evaluations of sweat and feces are confined to the electrolytes which can be measured precisely and accurately by sensitive methods. These two avenues of loss must be monitored if electrolyte balance studies are planned. Feces can be sampled with a reasonable degree of accuracy but unless the total sweat loss is accumulated, only spot checks on electrolytes can be done and balance studies based on food intake and feces, urine and sweat excretion will be approximations.

Since the analytical techniques and handling of the collected samples are quite similar for feces and sweat, they are both included in one section. Table 3-5 summarizes the information on sweat and feces.

FECES CONSTITUENT ANALYSES

1. Sodium: Method: Flame Photometry

   The technique is the same as for serum (see Blood Tests) but requires preliminary aqueous extraction or ashing of the sample.

   Volume: 1 gm
   Sensitivity:
   Precision: ±2%
   Accuracy: Highly accurate
   Preservation: Chemical
   Problems: None apparent. Chemical preservatives should be selected so as not to interfere with this determination.

2. Potassium: Method: Flame Photometry

   The technique is the same as for serum (see Blood Tests) but requires preliminary aqueous extraction or ashing of the sample.

   Volume: 1 gm
   Sensitivity:
   Precesion: ±2%
   Accuracy: Highly accurate
   Preservation: Chemical
   Problems: None apparent. Chemical preservatives should be selected so as not to interfere with this determination.

Table 3-5. Sweat Constituent Analyses

Sweat Constituent		Volume Required	Precision	Accuracy	Preservation Method
Sodium	Flame Photometry	0.1 ml	±2%	Highly accurate	Chemical
Potassium	Flame Photometry	0.1 ml	±2%	Highly accurate	Chemical
Chlorides	Chloride Titrator	1.0 ml	±2%	98.7-101.3% recovery of added chloride	Chemical
Calcium	Redox Titration of Oxalate	0.1-1.0 ml	±5%	Various errors tend to cancel each other out	Chemical

Feces Constituent Analyses

Feces Constituent					
Sodium	Flame Photometry	1 gm	±2%	Highly accurate	Chemical
Potassium	Flame Photometry	1 gm	±2%	Highly accurate	Chemical
Chlorides	Chloride Titrator	0.1 gm	±2%	98.7-101.3% recovery of added chloride	Chemical
Calcium	Redox Titration of Oxalate	1 gm	±5%	Various errors tend to cancel each other out	Chemical

3. Chlorides: Method: Chloride Titrator (conductimetric

Automatic conductimetric titration of chlorides.

Volume: 0.1 gm
Sensitivity: 0.25 μEq.
Precision: $\pm$2%
Accuracy: 98.7% - 101.3% recovery of added chloride.
Preservation: Chemical
Problems: Ashing of sample is not required. Chlorides can be determined directly on a fecal slurry. Chemical preservatives should be selected so as not to interfere with this determination.

4. Calcium: Method: Redox Titration of the Oxalate

Calcium is dissolved from an ashed sample of feces and precipitated as the oxalate which is then titrated with potassium permanganate or perchloratoceric acid.

Volume: 1 gm
Sensitivity:
Precision: $\pm$5%
Accuracy: Various errors tend to cancel each other out, and the results using this technique agree with results using other methods.
Preservation: Chemical
Problems: None apparent. Preliminary ashing should prevent interference from chemical preservatives.

SWEAT CONSTITUENT ANALYSES

1. Sodium: Method: Flame Photometry

See blood tests for description.

Volume: 0.1 ml
Sensitivity: Depends on type of instrument used.
Precision: $\pm$2%
Accuracy: Highly accurate
Problems: None apparent. Interference from chemical preservatives should not be a problem.

2. Potassium: Method: Flame Photometry

   See blood tests for description.

   Volume: 0.1 ml
   Sensitivity: Depends on type of instrument used.
   Precision: ±2%
   Accuracy: Highly accurate
   Problems: None apparent. Interference from chemical preservatives should not be a problem.

3. Chlorides: Method: Chloride Titrator (conductimetric)

   Automatic conductimetric titration of chlorides.

   Volume: 1.0 ml
   Sensitivity: 0.25 μEq.
   Precision: ±2%
   Accuracy: 98.7% -101.3% recovery of added chloride.
   Problems: None apparent.

4. Calcium: Method: Redox Titration of the Oxalate

   See feces tests for description and comments.

   Volume: 1.0 ml
   Sensitivity:
   Precision: ±5%
   Accuracy: Various errors tend to cancel each other out.
   Problems: Interference from chemical preservatives should not be a problem.

3.3.1 BIBLIOGRAPHY FOR FECES AND SWEAT ANALYSES

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SECTION 4
MICROORGANISMS

The microbiological aspect of this study is a problem unto itself. Unfortunately, there are no "normal" or "standard" values for numbers of genera of the microflora indigenous to man. The collection, preservation, and assay techniques are not as well defined as those for blood, urine, feces, and sweat, and are so closely interrelated that the discussion on microorganisms has been kept as a whole, rather than treating analysis, collection, and preservation separately.

Each individual has both on and within him his own personal indigenous population of microorganisms. Within, and on, a healthy person, the microfloral population is maintained in a state of dynamic equilibrium. Differences in genera, species, and/or serotypes of the members of this population occur between individuals and on the same individual when a change in environmental conditions occurs. These changes appear relevant to a consideration of the closed ecology found during long-term manned space flight.

The "normal" microbial population of a variety of individuals has been examined in the context of everyday life (1). The constitution of the microbial population of a given individual is the result of numerous interactions with the flora of other individuals, airborne microorganisms, and those found in or on food, water, clothing, and the myriad of inanimate objects with which one comes into contact during daily routines.

Isolation and confinement of a group of individuals changes the microbiological parameters qualitatively but not necessarily quantitatively. These changes, however, are of such magnitude (2, 3) that significant effects primarily related to fecal flora changes have been demonstrated in the few group isolation studies already performed. No clearly defined cause-effect relationships or methods of controlling these changes have been established. The available data from ground-based studies, which have shown the occurrence of significant

changes in the flora of confined groups of men, indicate a need for the assessment of any ecological changes during extended manned space missions. The constituent organisms of the type of closed ecological system under consideration will be men and microorganisms.

Instrumentation for physiological measurements is available, and equipment for clinical evaluations will be available. Thus, many of the biological phenomena exhibited in the ecosystem by the men during adaptation and/or reaction to long-term space flight will be measurable. Changes in the microbial portion of the ecosystem can also be measured. A number of factors must be considered in making this determination and are discussed below.

4.1 SAMPLING

An extremely important facet of the analytical protocol, regardless of whether the analyses are performed in-flight or post-flight, is sampling or recovery of microorganisms from the man and the spacecraft.

4.1.1 SAMPLING SITES

A recent report (4) suggests that for bacterial sampling, "the scalp, ear, eye, nose, throat, axilla, umbilicus, forearm, and anal area....can be sampled less frequently or eliminated from the monitoring schedule..." On a current GE contract, rhesus monkeys are being studied during long-term confinement. Microbiological sampling of the ear, nose, throat, mouth, axilla, groin, toes (hindfoot), scalp, and feces is required. No reduction in the number of body sites sampled is planned for the present phase of the work. However, after sufficient data has been obtained, the significance of the data from each body site will be determined. During several studies on confined groups of men, Enterobacteriaceae appeared on the glans penis and groin, and various fungi appeared on the glans penis, groin, and feet. Thus, these body sites should be included in a sampling regimen.

In addition to the men, sampling of the spacecraft will be required. During ground-based studies, beds, floors, communications equipment, refrigerator handle, transfer lock handle,

and eating, recreation, and personal hygiene areas were sampled. Other possible sampling sites are clothing (including pressure suits) and all knobs and surfaces which are expected to be in frequent contact with the skin or clothing of the crew members.

4.1.2 SAMPLING METHODS

With hypo-gravity as an environmental condition, settling plates cannot be used. Furthermore, the use of swabs in normal fashion is questionable because usually, after a sample is taken, a swab is immersed in a medium to insure that organisms in the interstices of the swab material will be recovered. Semi-solid agar is a possibility, but modification of present laboratory techniques will be needed to adapt swab techniques for in-flight use. Cotton and calcium alginate wool swabs are commonly used; it would be advantageous to include currently available synthetic materials such as polypropylene "wool" in a laboratory study of methods.

The impression method, using Rodac plates, has been widely accepted for surfaces. However, for sampling feces and most body sites, Rodac plates are unsuitable. Sterile sticky tape has been used for skin-surface sampling and could probably be adapted for swabbing the nostrils, ear canal, umbilicus, and groin by wrapping tape around an applicator stick or coating the applicator with an adhesive.

The technique for obtaining fecal samples will depend on the method of collecting feces. A prototype urine and feces collector with provision for sampling feces and urine has been built by the General Electric Company and is described in the section on Engineering Considerations. An aliquot of fecal slurry can be obtained via a sampling port. Handling of this slurry for plating would require equipment modified for use in weightlessness. However, since the sample is homogenized, the odds are in favor of getting a more representative sample. A serious drawback does exist, unfortunately, in that certain fecal organisms, especially anaerobes, may not withstand the time lag between defecation and culture of the sample, because of exposure to enriched oxygen atmosphere at less than atmospheric pressure,

lowering of temperature, or both; thus organisms of importance in the ecology of the space vehicle would either not be recovered at all or in numbers resulting from the survivors of an unknown original population. Here again, a laboratory study will be required to determine the better approach.

Plastic bags have been used on Gemini, and, esthetics aside, may be used on other vehicles. Devices for holding air permeable bags inside a molded toilet seat have been proposed, but the problems of pO_2 and ambient pressure effects on microorganisms must be weighed against the convenience. If plastic bags are to be used, a modified construction permitting samples to be taken without contaminating the cabin is certainly feasible.

Of great import in microbiological sampling are the media on which the samples will be grown. In all studies of the microflora of confined groups of men, several media have been used for each sample in order to increase the probability that all organisms recovered from a given sampling site will grow. In those tests where groups of four men were confined for periods of approximately four weeks, with a week each for pre-test and post-test sampling, the total time being six weeks, approximately 2500 cultures per man were made during sampling and an additional 50,000 per man were used during identification procedures. The volume of plates could be simply reduced by a factor of four by using quadrant-type petri plates which allow plating of four different media at one time. However, what portion of the cultures were made on plates and what portion in tubes is not known. Since a full gamut of media would be extremely difficult to use during space flight, a minimum complement of differential media is required.

No attempt has yet been reported to include the viruses and rickettsiae in studies on confined and isolated groups of men or animals. The men who have served as subjects in ground-based studies and the men chosen to be astronauts were, are, and will be subjected to a thorough medical examination. These men are all in that small portion of the population which could be said to be in excellent health. However, members of space vehicle

crew may harbor phages, adenoviruses, herpes, etc. even though no overt signs of an infection are discernible.

The rickettsiae are "not known to occur amphibiotically in man as they do in arthropods." (1) Since the crew members are healthy, and will be replaced prior to flight if any overt signs of an infection occur, there is little possibility that a rickettsial pathogen will be present.

It is suggested that culturing for viruses and rickettsiae be excluded from any on-board procedures for the first series of AAP flights. Post-flight analyses could conceivably show the presence of phages, but their importance to man and his indigenous flora is unknown.

Protozoa, while appearing quite often in the mouth, intestine, and genitourinary tract of man, are likewise not recommended for inclusion in an on-board culture routine at this time. As more knowledge of the nonpathogenic protozoa indigenous to man becomes available, sampling, culture, and identification methods, with emphasis on the latter two, may become less complex. At present, culture and identification of protozoa is hardly a simple matter, with separation of genera often requiring a greater knowledge of morphology than of culture techniques. For the shorter missions, provision can be made to bring back cultures for post-flight analyses, but for flights on the order of one hundred days, culture maintenance should be carefully worked out well in advance of any missions where protozoa culture return is planned.

4.2 ANALYSIS

In the manned studies to date, approximately 50 genera of bacteria, including aerobes and anaerobes, have been isolated, as well as representatives of at least 16 genera of fungi and several genera of protozoa. It is quite probable that this number of different genera will be encountered during a manned flight.

Since this is the era of automation, there is considerable interest in the automatic identification of bacteria. However, while the counting of microorganisms can be done automatically,

there is, as yet, no way to differentiate among bacteria, protozoa, and fungi, much less tell one species or genus from another in a mixed culture. Several techniques which may be of use in the future are briefly discussed below.

4.2.1 GAS CHROMATOGRAPHY

Detection and identification of bacteria by gas chromatography involves use of the gas chromatograph with highly sensitive detectors for the examination of bacterial products which are either volatile or can be converted to volatile derivatives (5). Many volatile compounds are synthesized by microorganisms during their growth and the sensitive detectors might be useful in determining the presence of small numbers of bacteria by detecting the formation of specific products. In view of the vast array of products which microorganisms can form, no one method for the extraction or chromatography on a single column of all the metabolites is possible.

Because of the sensitivity of the method, standardized culture techniques are mandatory. In addition, unless the population of bacteria obtained from a specimen is known to be homogeneous, or the distinctive chromatographic pattern is known not to be obscured by interfering organisms, pure culture techniques must first be employed. Furthermore, the number of bacterial signatures presently available for comparison with unknowns is insufficient to permit the use of the gas chromatograph as a laboratory tool. These factors mitigate against inclusion of gas chromatography for use at this time. However, because the methodology is still in the developmental stage, it is possible, and perhaps probable, that the development of the technique will continue to progress to the point where it could be used for long-term missions, either for post-flight analysis or for on-board use.

4.2.2 MASS SPECTROMETRY

Several groups have been investigating the identification of bacteria by mass spectrometry of their metabolic and pyrolysis products. The latter is an outgrowth of experiments on the relationship between structure and thermal stability of high temperature polymers. The limitations of gas chromatography are also applicable to mass spectrometry.

4.2.3 INFRARED SPECTROSCOPY

Identification of microorganisms by means of infrared spectra has been attempted by several investigators. However, samples must be prepared with extreme care from the same type of culture as the reference curve was prepared. The drawbacks of gas chromatography and mass spectrometry apply to infrared spectroscopy as well as the limitations inherent in an extremely sensitive procedure which is incompletely worked out.

None of the instrumental methods of analysis is sufficiently far along to be considered for laboratory use within the next two to three years. However, these approaches may well be developed into useful laboratory methods and should be taken into consideration during longer range planning.

4.2.4 FLUORESCENT ANTIBODY TECHNIQUE

This technique is mentioned because of its possible use both for in-flight and post-flight assays. A fluorescent compound (usually fluorescein isocyanate) is coupled to the specific gamma globulin with minimal loss in activity of the gamma globulin produced as response to a specific antigen. In this case, the antigen is a given species of bacteria. When the fluorescent antibody solution comes into contact with the antigenic bacterium, even in mixed cultures, the antigen is stained with fluorescent material visible in a microscope suitably modified for this purpose. The method has been found most useful for particulate antigens, such as bacteria. It is useful for identification of bacteria when a group of reference sera are available. With cells larger than bacteria, specific antigens within a cell may be localized.

Both microscope and staining equipment can be adapted for use in weightlessness. Major limitations of this method are: (1) it is primarily qualitative; (2) considerable a priori information must be available even if qualitative results are satisfactory; (3) cross reactions may occur. In clinical situations, fluorescent antibodies are used to check for the presence or absence of a limited number of pathogens.

If the appropriate sera can be prepared, the fluorescent antibody technique could be used for detection of marker and indicator organisms. Although the data would be qualitative, this method which has the sensitivity inherent in serological work could be used to check for transfer of marker organisms among crew members and to note the time of appearance or disappearance of a given organism from a sampling site.

4.2.5 MODIFICATION OF CONVENTIONAL METHODS

In view of the present status of instrumental methods of analysis, it is most likely that, for missions two or three years hence, conventional laboratory techniques, modified for use during weightlessness, will be used for assessment of ecological changes occurring during long-term space flights.

It is probable that major modifications will center around the use of liquid media. Feasibility studies performed by General Electric show that proper choice of culture tube geometry will keep broth within a conical section of the tube. (See Figure 6-18.) However, the insertion of a swab into the tube must be done with extreme care to prevent escape of liquid media to the cabin. Several concepts which would prevent escape of liquid during manipulations have been considered during an in-house study of sampling, analysis, and preservation of biological materials, including microorganisms. If liquid media are used, transfer of aliquots to other solid or liquid media for subculturing can be accomplished with syringes. As stated in the section on sampling, though, a conventional protocol utilizing large numbers of culture vessels and different media is too unwieldy for use during flight.

4.3 MEDIA

A variety of media will be required for on-board sampling and preservation of cultures. One of the problems that will become greater, as missions become longer and/or involve a greater extent of on-board analysis, is media storage. By using small, quandrant type petri plates the number of plates and the volume needed for storage can be decreased. For missions of up to 4 to 6 weeks, refrigerated storage of plates and tubes will suffice. For

longer missions, hermetically sealed sterile containers should be considered for maintenance of sterility and moisture content. New media should also be developed for missions of 45 to 135 days. Solubilized, sterile dry media already placed in tubes and sterile agar "sponges" in petri plates to which sterile water could be added aseptically, are possibilities which are already feasible from an equipment standpoint.

In addition, new formulations should be considered. A "transport" medium (6, 7) has been developed and is being field tested. This medium was developed specifically for Mycobacteria but will also support Shigella, Salmonella, and Vibrio for extended periods of time at 20° to 31°C. The development of media which could be used for the support of microorganisms during "storage" and which would ensure a known percent recovery seems a fruitful area for further study.

4.4 PRESERVATION OF SAMPLES FOR POST-FLIGHT ANALYSIS

Initial cultures of certain samples, i.e., swabs and feces, can be made in liquid media during flight. However, for both in-flight and post-flight analyses, subcultures must be made. For those samples to be returned, the subcultures, which will be identified in a laboratory on the ground should be made in liquid and solid media in order to enhance the recovery of microorganisms in the sample.

It is recommended that cultures to be returned be made in duplicate for both incubation and refrigeration. Lyophilization of samples is not recommended for preservation of microbiological samples. (Any protozoa present will be adversely affected by this treatment.) Although recovery of organisms from lyophilized pure cultures is on the order of 70 -95%, an assessment of the microbial ecology of a spececraft would be incomplete because those organisms labile to lyophilization will not survive.

4.4.1 INCUBATION

For the majority of the microorganisms anticipated, an incubation temperature of 37°C will suffice. Few, if any, thermophils are expected but will probably grow at a slower

rate at 37°C. The organisms recovered from the cabin, other than those from the astronauts or ground crew, will probably be common airborne contaminants which will also grow at 37°C, but it may be desirable to include a 30°C incubator to enhance the recovery of those organisms with optimum growth temperatures closer to 30°C than to 37°C.

4.4.2 REFRIGERATION

The transport medium mentioned above shows promise in keeping certain microorganisms viable at 4°C. Further work is necessary, of course, but this avenue should be explored further. Since refrigeration equipment will be required for maintaining frozen and/or cooled samples of serum and urine, it may be simpler to refrigerate at least a major fraction of the microfloral cultures than to incubate them.

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SECTION 5

PRESERVATION TECHNIQUES

5.1 SUMMARY

Each of the six basic preservation techniques studied have several approaches by which they can be accomplished. A detailed systems engineering analysis of each approach is given in this section and the most promising approach to each technique is summarized below and illustrated in Figure 5-1. A weight, power, and volume comparison of the techniques is shown in Table 5-1 and the engineering preference based on weight is also listed.

Weight was used as the most important selection criterion because nearly 1500 pounds of booster thrust is required to put each pound of payload into an extended mission orbit or flight. Power is also very important because of the weight required to produce each watt of electrical energy; however, the weight/power penalty may vary between 0.5 and 2.0 pounds per watt, depending on vehicle and mission parameters. The higher penalty does not affect the order of engineering preference. Consequently, power cannot be used as a major selection criterion until further definition is made of the vehicle power system.

5.1.1 CHEMICAL

First engineering choice - Appropriate chemical preservatives are prepacked in each biological sample container. This approach negates the need for chemical dispensing mechanisms and will permit color coding such that red sample containers have a preservative for blood, yellow containers a preservative for urine, etc. Chemical preservation offers the lightest launch weight and smallest launch volume technique for sample preservation. No electrical power is required.

5.1.2 REFRIGERATION (CHILLING)

Second Engineering Choice - A space radiator heat sink and a heat transport medium as utilized to cool the biological samples. This approach does not require expendable refrigerants and requires only small amounts of electrical power. A proper space heat sink can be

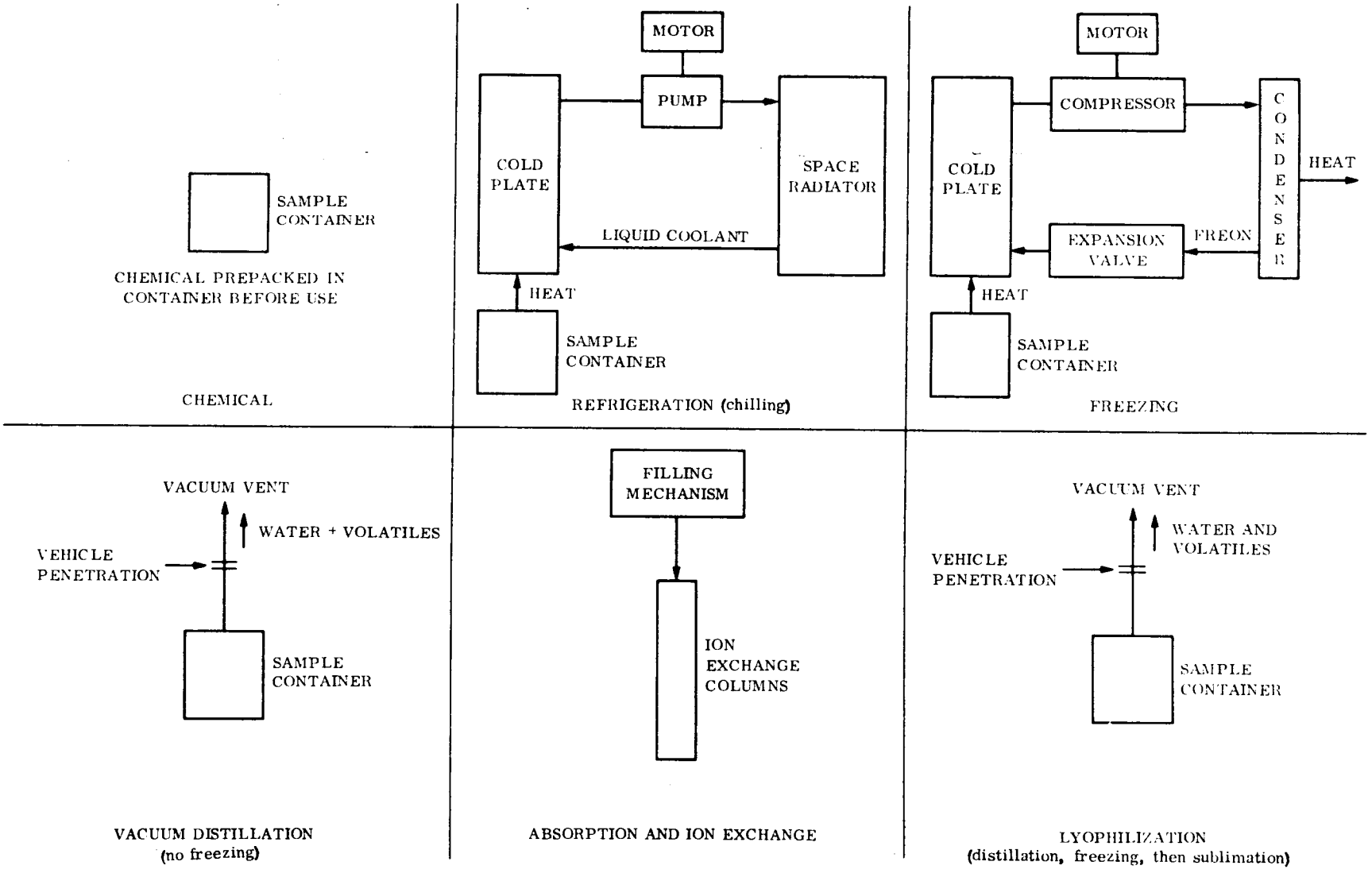


Figure 5-1. Selected Approach to Each Preservation Technique

Table 5-1. Comparison of Preservation Techniques, Weight, Volume and Power for 1000-10 Gram Samples

ENGINEERING PREFERENCE*	PRESERVATION TECHNIQUE	LAUNCH WEIGHT (lbs.)	RETURN** WEIGHT (lbs.)	LAUNCH VOLUME (ft^3)	RETURN** VOLUME (ft^3)	ELECTRICAL POWER (watts)	STATE OF DEVELOPMENT
1	Chemical	2.8	24.8	0.2	0.8	----	Prototype
2	Refrigeration @ 35°F (1.6°C)	8.0	26.8(1)	1.4	1.4(1)	3.2	Prototype
3	Freezing @ -40°F (-40°C)	13.0	35.0(2)	1.9	1.9(2)	24.0	Laboratory
4	Vacuum Distillation	28.0	11.0(3)	2.0	0.3(3)	----	Prototype
5	Lyophilization	45.0	11.0(3)	2.6	0.3(3)	----	Prototype
6	Absorption and Ion Exchange	----	----	----	----	----	Laboratory

* Based on weight and state of development.

** The return weight and volume is that of the samples and container returned via the ferry vehicle to earth.

(1) Not including radiator and pump (3.2 lb.).

(2) Including Freon vapor cycle unit.

(3) Assuming 90% loss of sample weight and volume. Includes bag weight and volume. The drying chambers remain in the launch vehicle and are not returned. This accounts for the majority of the launch weight and volume.

achieved in nearly any spacecraft attitude and orbit. Refrigeration has the second lowest launch weight and volume requirement and requires only a small amount of electrical power for the coolant pump.

5.1.3 FREEZING

Third Engineering Choice - A Freon vapor cycle unit is used to freeze the biological samples. This approach does not require expendable refrigerants or a special heat sink and it utilizes only a moderate amount of electrical power. However, if a proper spacecraft attitude and orbit is provided, then a system similar to that used for refrigeration is recommended. Preservation by freezing has the third lowest launch weight and volume, but requires the most electrical power for the Freon compressor.

5.1.4 VACUUM DISTILLATION (DRYING)

Fourth Engineering Choice - Specially designed sample containers are used to retain the sample solids while venting the water and other volatiles to space vacuum. This approach is very simple and utilizes minimal electrical power. Vacuum distillation, as a preservation method, has the second highest launch weight and volume requirement because of the large number of drying chambers required.

5.1.5 LYOPHILIZATION

Fifth Engineering Choice - A specially designed sample container is used which permits freezing and sublimation of the biological sample with the sample solids being retained in a porous material. This approach is very simple and requires minimal electrical power; however, the heat transport mechanism is a complicating factor. Lyophilization has the highest launch weight and volume requirement because of the slow rate of sublimation and the resulting large number of chambers required to dry the samples.

5.1.6 ADSORPTION AND ION EXCHANGE

Sixth Engineering Choice - A mechanical pump is used to force a sample into the ion exchange columns. It is doubtful if this preservation technique will be used because of the large amounts

of ion exchange materials required and the questionable sample extraction techniques. No weights, volumes, or powers were determined for this technique.

5.2 SELECTION AND APPROACH TO EACH PRESERVATION TECHNIQUE

There are several engineering approaches to each preservation technique, all of which are compatible with space vehicle environments, e.g., shock, vibration, zero gravity, etc. Each approach is rated for several significant items of equal importance. This gives a crude (first cut) means of comparison of the different approaches to each preservation technique. The methods that appear to be the most promising are indicated by an asterisk (*) in the following tables.

Note

This type comparison is used only to select the best approach to each preservation technique and cannot be used to compare the techniques. Comparison of techniques (Table 5-1) can be made only after determination of the weight, volume, and power requirement for the selected approaches.

Definition of Terms

Electrical Power Required - is the average power required by the system. No weight penalty for the power supply system is used because it is not sufficiently defined.

Weight - is the total system weight including sample containers, storage modules, mechanical and electrical devices, expendables, etc.

Complexity and Reliability- are used as antonymous terms. Complexity/reliability is a gross assessment of the number of moving parts, design complexity and resulting overall reliability of the system.

Expendables - are any materials lost from the vehicle or consumed within the vehicle to achieve preservation. Chemical preservatives are considered an expendable along with refrigerants which are evaporated to space in order to obtain cooling.

Vehicle Penetrations - are any openings in the vehicle cabin structure to permit venting of gases and/or liquids to space or to provide a vacuum to internal components. Any penetration is a cause for concern because it is another possible source for leakage and it detracts from the structural integrity of the pressure vessel (cabin). Typically, the stress concentration around a hole in a plate under tension will be three times that of a non-penetrated plate. All known manned and animal flights to date have used vehicle penetrations of some type; however, penetrations should be minimized as a safety measure.

Ease of Operation - is the amount of manual dexterity and skill required by the astronaut to preserve the biological samples. A 10 rating will require little or no training and applies to simple tasks, comparable in difficulty to sharpening a pencil or shifting gears in a car. The 1 rating applies to more intricate tasks which require dexterity and attention to detail such as installing and setting the gap for a set of points on an automobile engine.

Safety - includes consideration of any possible toxic, fire, or explosion hazard inherent in the system. All systems use plastic sample storage containers which are a possible fire hazard. Laboratory tests are required to define the hazard and to recommend the best plastic for this application.

Freon used to pressurize vessels and for the Freon vapor cycle unit are not very toxic in themselves but if a catalytic burner is used for normal vehicle toxic gas control (e.g. for CO), a leakage of Freon into the cabin atmosphere may produce phosgene (a very toxic gas) when it is passed through the vehicle toxic gas control system. For this reason FreonC318 is recommended for use in pressurizing vessels, because it has been shown not to produce toxic gases when passed through a low temperature catalytic oxidizer such as Hopcalite. The Freon for the vapor cycle unit is not as easily handled since Freon 12 or 22 must be used because of the thermal characteristics, and these Freons will produce phosgene in the vehicle toxic gas control system. Fortunately the Freons are easily detected by halogen sensors; the toxic gas control system can be shut off and the cabin may be purged or artificial zeolite may be used to adsorb the Freon should leakage occur.

Obviously the safety aspects of the various preservation approaches require a much more detailed study than is set forth here. A complete study of the entire vehicle is the only way to ensure adequate safety procedures. Safety hazard listings for individual subsystems are helpful but do not tell the whole story. For example, a leakage of Freon from the vapor cycle unit is not hazardous by itself but when other vehicle systems are involved a definite toxic gas hazard exists.

5.2.1 CHEMICAL

Several approaches to the chemical preservation technique are shown in Table 5-2.

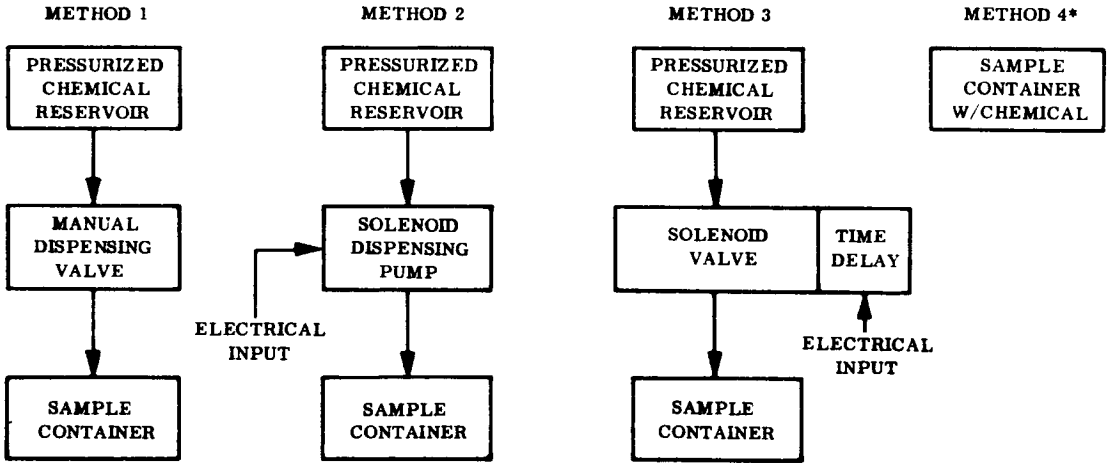
Approach No. 1 - utilizes a pressurized chemical reservoir with a manual dispensing valve to meter the chemical into the sample container. This method is mechanically simple and the amounts dispensed can be accurately controlled.

Approach No. 2 - utilizes a pressurized chemical reservoir with a solenoid activated dispensing pump to meter the chemical into the sample container. This method may be semi-automated and the amounts dispensed can be very accurately controlled. A similar device is used in the Biosatellite Program for injecting heparin into the blood stream of a monkey.

Approach No. 3 - utilizes a pressurized chemical reservoir with a solenoid valve and time delay circuitry to hold the valve open for set periods. The valve automatically dispenses a calibrated amount of chemical into the sample container. A similar device is used in the Biosatellite Program to dispense disinfectant into a feces collector.

Approach No. 4 - is by far the simplest method and is therefore recommended (see Table 5-2). An additional benefit derived from this method of storing the chemical in the sample container permits different chemical preservatives to be used without additional dispensing mechanisms. For example, sample containers colored red would contain a preservative for blood, yellow containers a preservative for urine, brown for feces, etc.

Table 5-2. Preservation by Chemical Methods



ITEM	RATING			
	METHOD 1	METHOD 2	METHOD 3	METHOD 4
ELECTRICAL POWER REQUIRED	10	1	5	10
WEIGHT	5	1	8	10
COMPLEXITY/RELIABILITY	8	5	5	10
EXPENDABLES	1	1	1	1
VEHICLE PENETRATIONS	10	10	10	10
EASE OF OPERATION	1	5	5	10
SAFETY	5 (a)	1 (a) (b)	1 (a) (b)	10 (c)
TOTAL	40	24	35	61*

NOTE: ALL ITEMS HAVE EQUAL IMPORTANCE

RATING: 10= BEST
1= WORST

*SELECTED

(a) PRESSURE VESSEL HAZARD

(b) SOLENOID JAMMING HAZARD

(c) PRESERVATIVE LEAKAGE HAZARD

5.2.2 REFRIGERATION (CHILLING)

and

5.2.3 FREEZING

Refrigeration is defined as chilling or cooling the sample but not freezing. The several system approaches for either refrigeration or freezing preservation have the same basic components as shown in Table 5-3.

Approach No. 1 utilizes a Freon vapor cycle unit to cool and/or freeze the sample. This is the recommended approach for freezing because of the moderate power requirement, no vehicle penetration required, and the entire sample is retained. The amount of cooling required by a biological sample varies for different fluids; however, the thermodynamic characteristics of these fluids approximates water. The temperature enthalpy characteristics for water are listed in Table 5-4. The cooling requirements are then calculated as follows:

$$\text{Cooling } Q = \frac{W \text{ (grams)}}{454 \text{ grams/lb}} \times \frac{\Delta h \text{ (BTU/lb)}}{3.415 \text{ BUT/watt-hr.}} \times \frac{60 \text{ min.}}{\text{min hr}} = \text{watts}$$

Example: For a 10 gram sample cooled from $100^{\circ}F$ ($37.8^{\circ}C$) to $-4^{\circ}F$ ($-20^{\circ}C$) in five minutes:

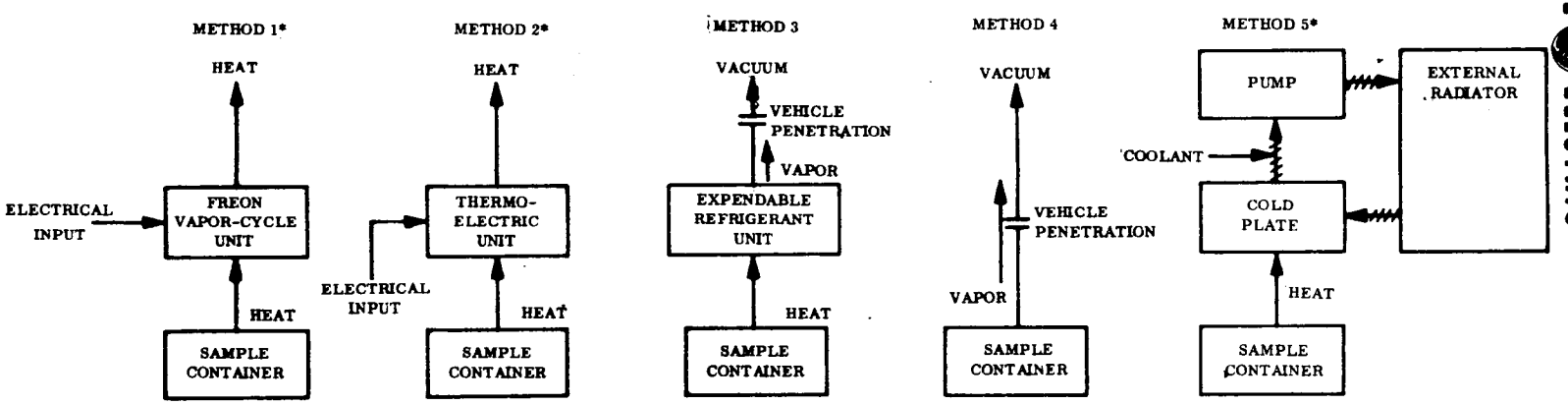
$$Q = \frac{10}{454} \times \frac{225}{3.415} \times \frac{60}{5} = 17.42 \text{ watts cooling required.}$$

Heat gains (leakage) through insulation and door openings is assumed to be 0.01 watts per gram sample per $^{\circ}F$ temperature difference between freezer and ambient temperature.

Example: For a 10 gram sample at $-4^{\circ}F$ ($-20^{\circ}C$) with an ambient temperature of $75^{\circ}F$ ($24.8^{\circ}C$):

$$\text{Gains} = 10 \times \left[75 - (-4^{\circ}F)\right] \times 0.01 = 7.9 \text{ watts gain}$$

Table 5-3. Preservation by Refrigeration or Freezing



ITEM	RATING				
	METHOD 1	METHOD 2	METHOD 3	METHOD 4	METHOD 5
ELECTRICAL POWER REQUIRED	5	1	10	10	9
WEIGHT	5	5	1	10	7
COMPLEXITY/RELIABILITY	3	8	5	7	4
EXPENDABLES	10	10	1	10 SAMPLE LIQUID	10
VEHICLE PENETRATIONS	10	10	1	1	10
EASE OF OPERATION	10	10	10	1	10
SAFETY	1 (a)	10	1 (a)	5 (b)	5 (a)
TOTAL	44 * (c)	54 * (d)	29	44	55 * (e)

NOTE: ALL ITEMS HAVE EQUAL IMPORTANCE.
RATING: 10 = BEST
1 = WORST
*SELECTED

(a) GAS OR LIQUID LEAKAGE HAZARD

(b) VACUUM SEAL HAZARD

(c) SELECTED FOR FREEZING

(d) ELIMINATED BY POWER REQUIREMENT

(e) SELECTED FOR REFRIGERATION

Table 5-4. Temperature - Enthalpy Chart for Water

TEMPERATURE	h ENTHALPY (BTU/LB)	Δh DELTA ENTHALPY (BTU/LB)
Initial 100°F (37.8°C)	h_f = 60	
40 °F (4°C)	h_f = 8	Δh = 52
32°F (0°C)	h_f = 0	Δh = 60
32°F (0°C)	h_s = -144	Δh_{fs} = 204
14°F (-10°C)	h_s = -155	Δh_{fs} = 215
-4°F (-20°C)	h_s = -165	Δh_{fs} = 225
-22°F (-30°C)	h_s = -175	Δh_{fs} = 235
-40°F (-40°C)	h_s = -185	Δh_{fs} = 245

Total cooling requirement is then:

Cooling sample	17.42 watts
Heat gains	7.9
	25.32

The Freon-vapor cycle freezer is assumed to have a coefficient of performance (COP) of 4 with a drive motor efficiency of 50 percent; therefore, the overall COP of the machine is 2. This means that for every watt of power input to the machine, 2 watts of cooling are achieved. Therefore, the 25.32 watt cooling requirement necessitates an electrical input of 12.66 watts.

Figure 5-2 illustrates several curves for various sample sizes and temperature versus electrical power input for a freezer of the above type. After initial freezing of the sample, the cooling requirement will decrease to that required to maintain the temperature. If the freezer is designed with a sufficiently large thermal mass, it will be possible to decrease the peak power requirement by dissipating the initial sample heat to the mass of the freezer.

Approach No. 2 utilizes a thermoelectric unit to cool and/or freeze the sample. This method proved to have a good rating when compared to other approaches in Table 5-3; however, further study limits this high rating to small samples. Figure 5-3 shows that the electrical power requirement becomes prohibitive for large samples. A similar system was used to cool small frog egg experiments for Gemini 8 and 12.

Approach No. 3 utilizes an expendable refrigerant such as alcohol to cool and/or freeze the sample. The alcohol is evaporated at low pressures (reference to spatial vacuum) which removes heat from the sample. The pressure, and thus the temperature, is controlled by an absolute pressure regulator. This method was initially studied for the Biosatellite Program (1) where electrical power was at a premium. Subsequently, the collection hardware has been fabricated and tested as part of a company funded program.

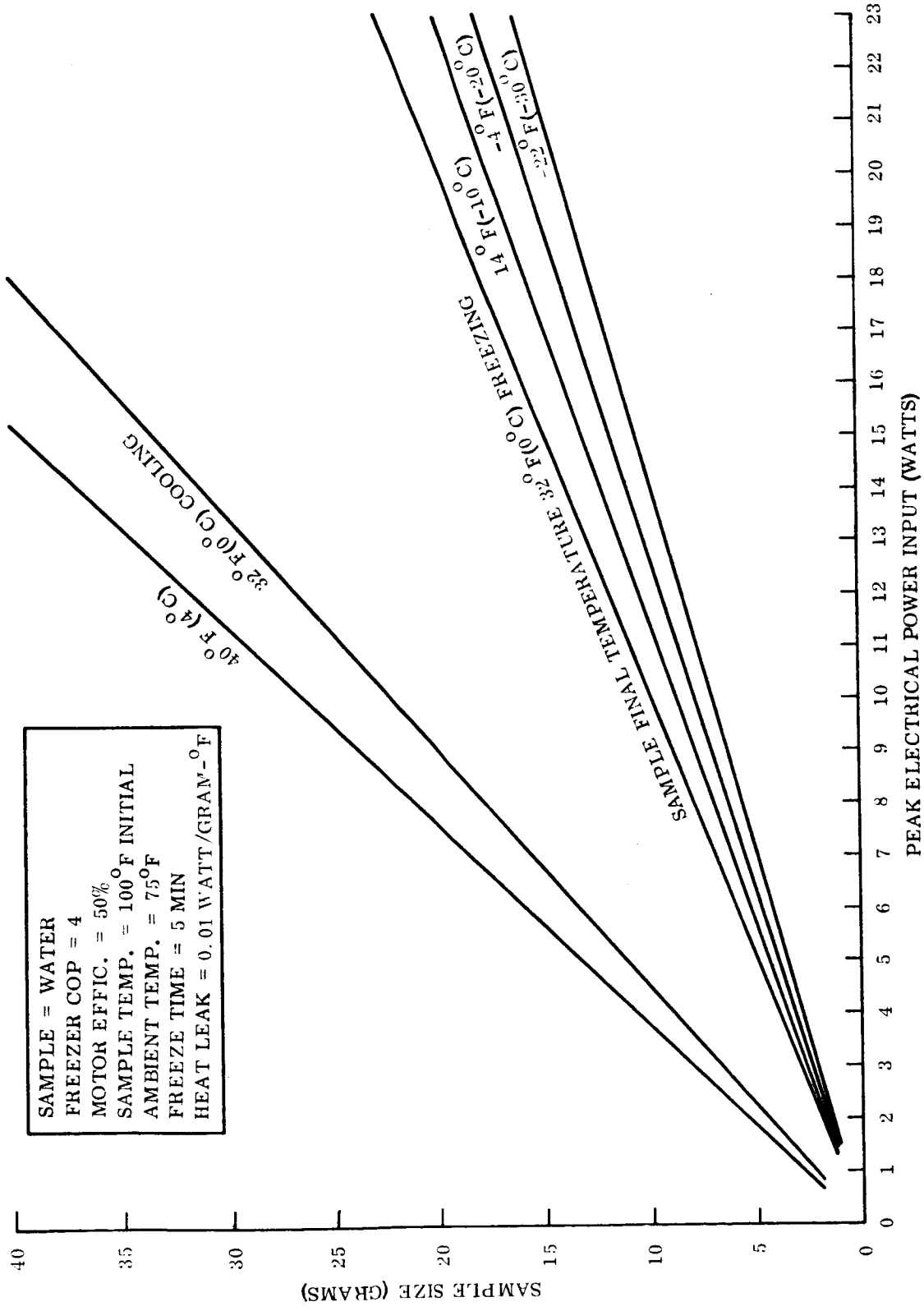


Figure 5-2. Sample Size versus Power Input for Freon Vapor Cycle Unit

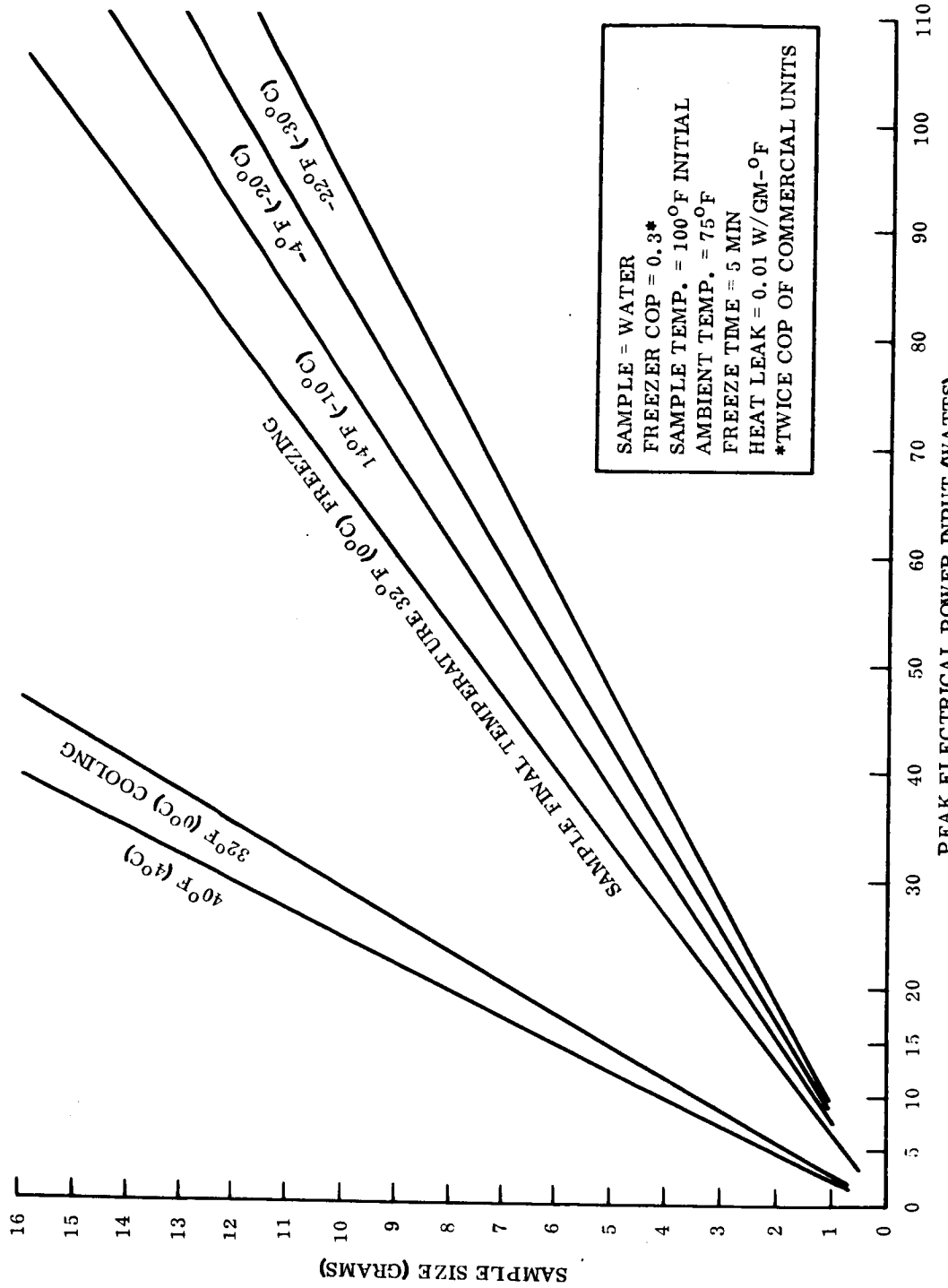


Figure 5-3. Sample Size versus Power Input for Thermoelectric Cooling Unit

Approach No. 4 utilizes the sample volatile liquid as the expendable refrigerant. As the sample is exposed to low pressure, the water is vaporized by removing heat from itself. Thus the sample may be cooled or even frozen. A method to retain the urine solids during boiling is achieved by porous materials which retain the solid while permitting the vapor to pass through. Basic studies and experimentations on this method have been conducted by GE (3).

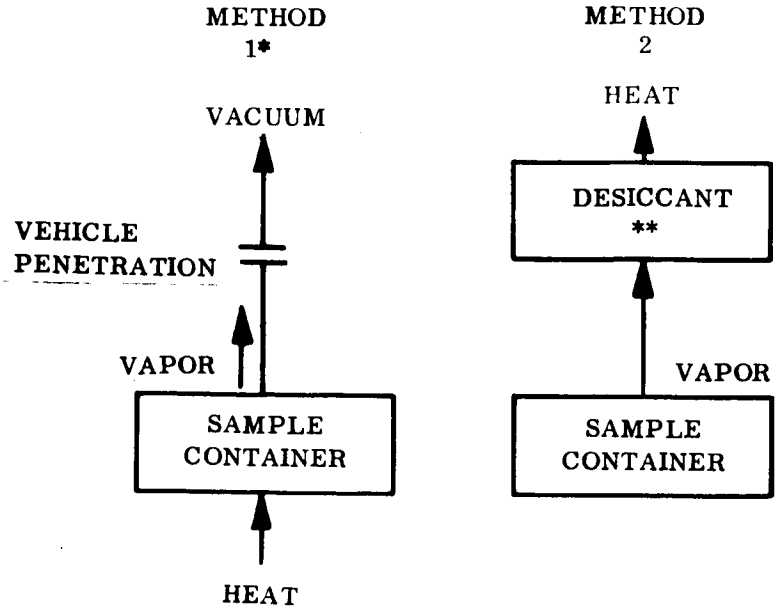
Approach No. 5 utilizes a space radiator to reject sample heat to the spatial heat sink. The sample is attached to a cool plate which is cooled by a liquid heat transport medium such as "Coolanol" or aqueous ethylene glycol. The coolant is pumped to a radiator which, when shielded from the sun and the albedo from the earth, moon, etc., will see a heat sink temperature $-460^{o}F$ ($-273^{o}C$) (4). Proper coating of the radiator surface, such that high emissivity and low absorptivity are achieved, will permit sufficient cooling of the coolant for sample refrigeration purposes without orientation of the radiator in a near earth orbit. This is the recommended approach for sample refrigeration. If proper orientation of the radiator can be assured, this is also the recommended approach for sample freezing. Much more thermal analysis will be required for specific missions.

5.2.4 VACUUM DISTILLATION

Approach No. 1 of the vacuum distillation technique for sample preservation is basically the same as Approach No. 4 of the Refrigeration and Freezing technique. The only difference is that the sample container is designed to conduct heat rather than to be an insulator. This permits rapid distillation of the sample with little cooling effect. Again the sample solids are retained by a porous material while the volatiles are vented to space vacuum (3). See Table 5-5.

Approach No. 2 utilizes a desiccant such as silica gel or molecular sieves to dry the sample. This is essentially the same as venting the sample to space vacuum, except that the desiccant acts as a "getter" for only the water vapor and the remaining gases tend to modulate the boiling to a less violent rate. The desiccant may be regenerated by venting it to space vacuum

Table 5-5. Preservation by Vacuum Distillation



ITEM	RATING	
	METHOD 1	METHOD 2
ELECTRICAL POWER REQUIRED	10	10
WEIGHT	10	3
COMPLEXITY/RELIABILITY	7	7
EXPENDABLES	10 SAMPLE LIQUID	1
VEHICLE PENETRATION	1	10
EASE OF OPERATION	5	5
SAFETY	5 (a)	10
TOTAL	48*	46

NOTE: ALL ITEMS HAVE EQUAL IMPORTANCE.

RATING: 10 = BEST
1 = WORST

*SELECTED

**IN THIS CASE THE DESICCANT ACTS AS A WATER VAPOR PUMP AND CANNOT BE ACCURATELY CALLED VACUUM DISTILLATION.

(a) VACUUM SEAL HAZARD

and transferring heat to it from the ambient environment. Trade-offs are required to determine whether it is better to regenerate or to just dispose of the desiccant.

Vacuum distillation Approach No. 1 is recommended since no expendables or complicated mechanism are required.

5.2.5 ADSORPTION AND ION EXCHANGE

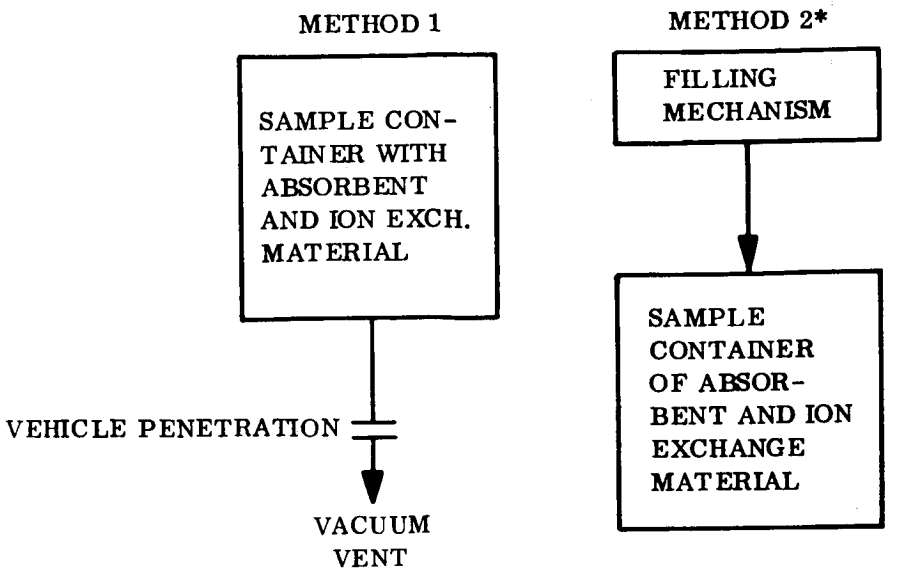
The adsorption and ion exchange technique of preservation of samples is analogous to liquid purification. The main difference is that the residue is of more interest than the effluent; however, the desire to remove all the impurities from the effluent is of equal importance. Much work has been done in this area for recovery of water from sea water and urine (7-15). Two system approaches are shown in Table 5-6.

Approach No. 1 utilizes a relatively simple system with a tube column packed with different adsorbents and ion exchange resins. The sample is injected at one end of the tube and is drawn through the column by a pressure differential induced by venting the other end of the column to spatial vacuum. The effluent is either collected in a sponge or vented to space. Approach No. 2 utilize the same column design as Approach No. 1; however, the sample is forced through the column by a manually operated piston filling mechanism or pressure from the sample collective device. The engineering rating, (Table 5-6) of the adsorption and ion exchange preservation technique is lower than several other techniques. This is mainly due to the amount of expendable materials and the weight. Also, the problems in maintaining the sterility of the sample without refrigeration are formidable; with refrigeration, weight and volume become prohibitive. Use of chemical preservatives with adsorbents is an area requiring considerable research. In addition, the variety of ion exchange resins required for preservation of organic and inorganic compounds plus their lack of true specificity strongly mitigates against their further consideration.

5.2.6 LYOPHILIZATION

Lyophilization (16-22) is a rather unique preservation technique from an engineering standpoint. Initially the sample is rapidly frozen; then, the sample is subjected to a very low pressure and

Table 5-6. Preservation by Adsorption and Ion Exchange



ITEM	RATING	
	METHOD 1	METHOD 2
ELECTRICAL POWER REQ'D	10	10
WEIGHT	3	1
COMPLEXITY/RELIABILITY	5	4
EXPENDABLES	1	1
VEHICLE PENETRATIONS	1	10
EASE OF OPERATION	5	1
SAFETY	5 (a)	10
TOTAL	30	37*

NOTE: ALL ITEMS HAVE EQUAL IMPORTANCE

RATING: 10 = BEST
1 = WORST

*SELECTED

(a) VACUUM SEAL HAZARD

the volatiles are sublimed. The sublimation process, however, requires the addition of heat so that both cooling and heating of the sample are required. Also, as the sample volatiles sublime, the volume decreases and sample contact with the heat exchange surface may be lost. This will result in an erratic sublimation rate. Previous designs of this type (4), utilize a mechanical force (spring) to maintain the sample in contact with the heat exchange surface. Possibly retaining the sample in a porous metal block, so that the small portions of the sample are always in close proximity to the heat exchange surface, will also resolve the problem (3). Three system approaches are shown in Table 5-7.

Approach No. 1 utilizes a cooling unit, either Freon vapor cycle or thermoelectric, to freeze the sample. When the sample is subjected to low pressures, the cooling unit is used as a heat pump to supply the heat of sublimation. Approach No. 2 utilizes the volatiles in the sample to evaporate at a low pressure and freeze the sample. After freezing, enough heat is added from the ambient environment or electrical heat to permit sublimation. Approach No. 3 utilizes a desiccant as a vapor pump to permit freezing and then sublimation of the sample. Approach No. 2 shows a superior rating for this preservation technique (see Table 5-7).

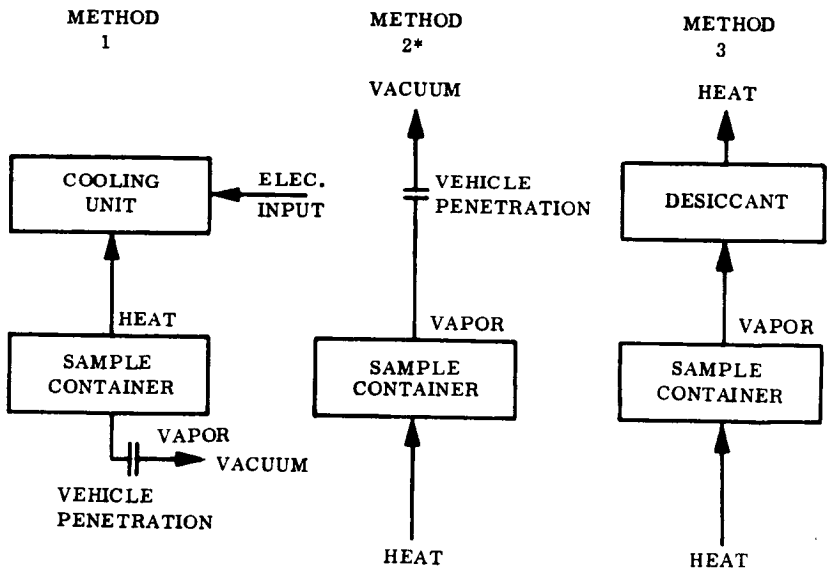
5.3 EVALUATION OF PRESERVATION TECHNIQUES

The several selected preservation techniques are discussed in detail in this section. Weight, volume and power determination are also made so that the best preservation technique can be selected for specific mission requirements. All samples should be identified by a tag on which is automatically printed the subject's name, the time, the date and the type of sample.

5.3.1 CHEMICAL

The selected approach for chemical preservation is to initially store the preservative in the sample container, possibly with different color containers containing preservatives for different biological samples. If a bactericide is required as an added precaution, the container material, such as polyvinyl chloride, will incorporate a biocidal compound with the plasticizer. Assuming that each sampling bag weighs 0.125 grams per gram of sample, including

Table 5-7. Preservation by Lyophilization



ITEM	RATING		
	METHOD 1	METHOD 2	METHOD 3
ELECTRICAL POWER REQ'D	5	10	10
WEIGHT	5	10	3
COMPLEXITY/RELIABILITY	3	5	5
EXPENDABLES	10 SAMPLE LIQUID	10 SAMPLE LIQUID	1
VEHICLE PENETRATIONS	1	1	10
EASE OF OPERATION	5	5	5
SAFETY	5 (a)	5 (a)	10
TOTAL	34	46*	44

NOTE: ALL ITEMS HAVE EQUAL IMPORTANCE.

RATING: 10 = BEST
1 = WORST

*SELECTED

(a) VACUUM SEAL HAZARD

preservative, and that the package volume of each unused sample bag is 0.5 cc per gram of sample, the initial weight and volume of the chemical preservation technique will be as illustrated in Figure 5-4. After sampling, assuming an overall 50 percent packaging efficiency, (Specific gravity = 0.5), the final weight and volume of the chemical preservation technique is illustrated in Figure 5-5. No electrical power is required.

The sample bags are initially sealed to retain the preservative. The seal is broken to inject the sample and the bag is resealed. Possibly a thermoplastic bag will be used for this purpose so that it can be heat sealed, or the bag will have a self-sealing septum, or a sealant such as RTV (Room Temperature Vulcanizing) silicone rubber could be used to reseal the sample container.

The sealed sample container is then stored in the previously emptied food storage area. No special storage is required except for easy removal through the airlock of all sample storage modules during resupply.

5.3.2 REFRIGERATION

Refrigeration or chilling of biological samples can best be achieved by rejecting the sample heat to a space heat sink (see Figure 5-6). In normal near earth orbit, the space heat sink temperature will average approximately $-100^{o}F$ ($-73.3^{\circ}C$) with a proper coating for high emissivity and low absorptivity. Based on past experience (4), a coolant temperature of approximately $-10^{o}F$ ($-24.6\ ^{o}C$) is achieved with this arrangement. Consequently, sample chilling to as low as 0 to $+10^{o}F$ (-18.2 to $-13.5^{o}C$) may be achieved with this preservation technique. Obviously, as the mission changes from near earth to deep space, lower sink temperatures will be achieved so that much lower sample preservation temperatures will be possible. In that case, this approach will also be recommended for preservation by freezing.

The weight, physical size and power requirement for the space radiator refrigerator unit is mainly based on the characteristics of the radiator, coolant pump and insulated sample storage box.

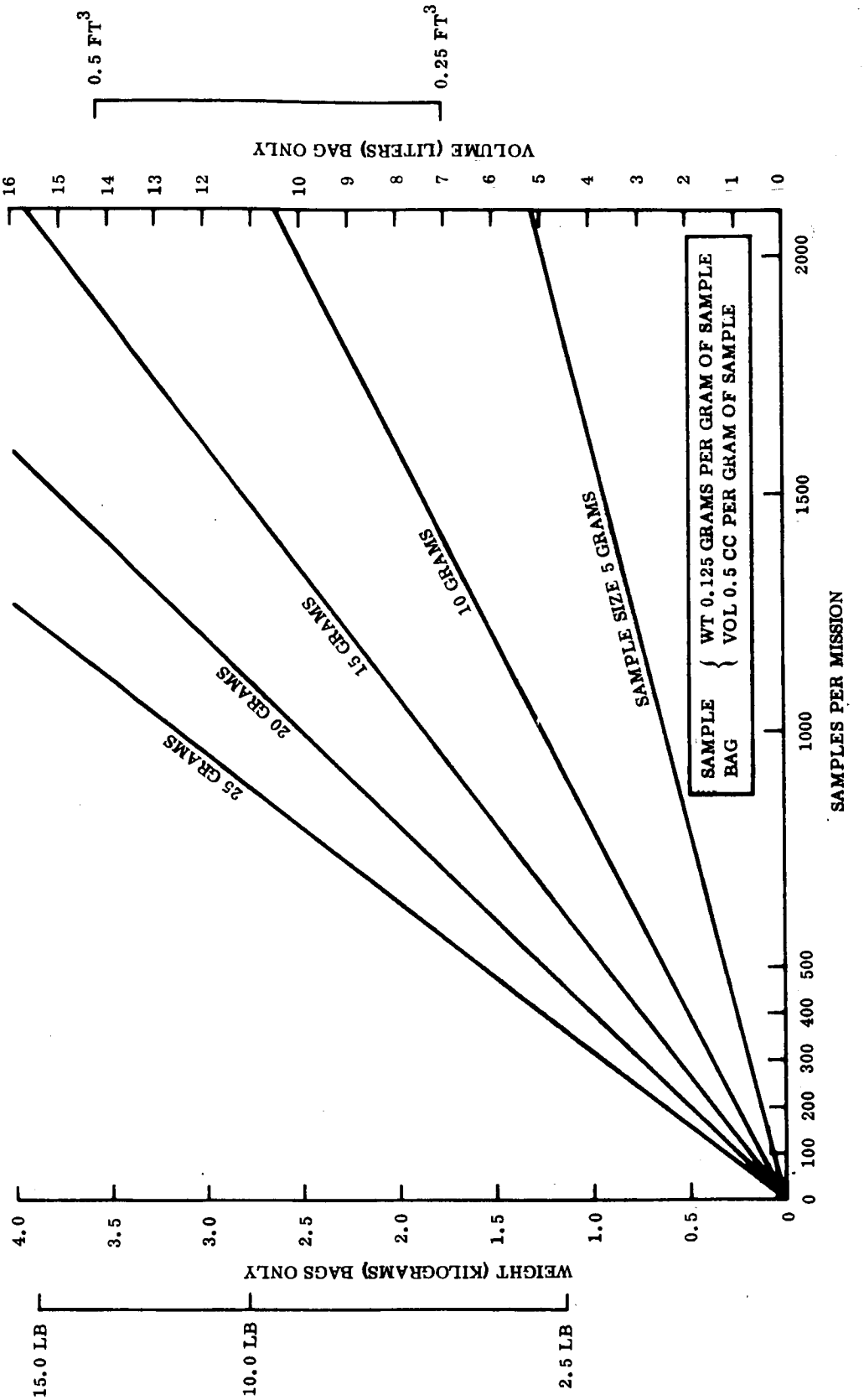


Figure 5-4. Initial (Empty) Weight and Volume for Chemical Preservation

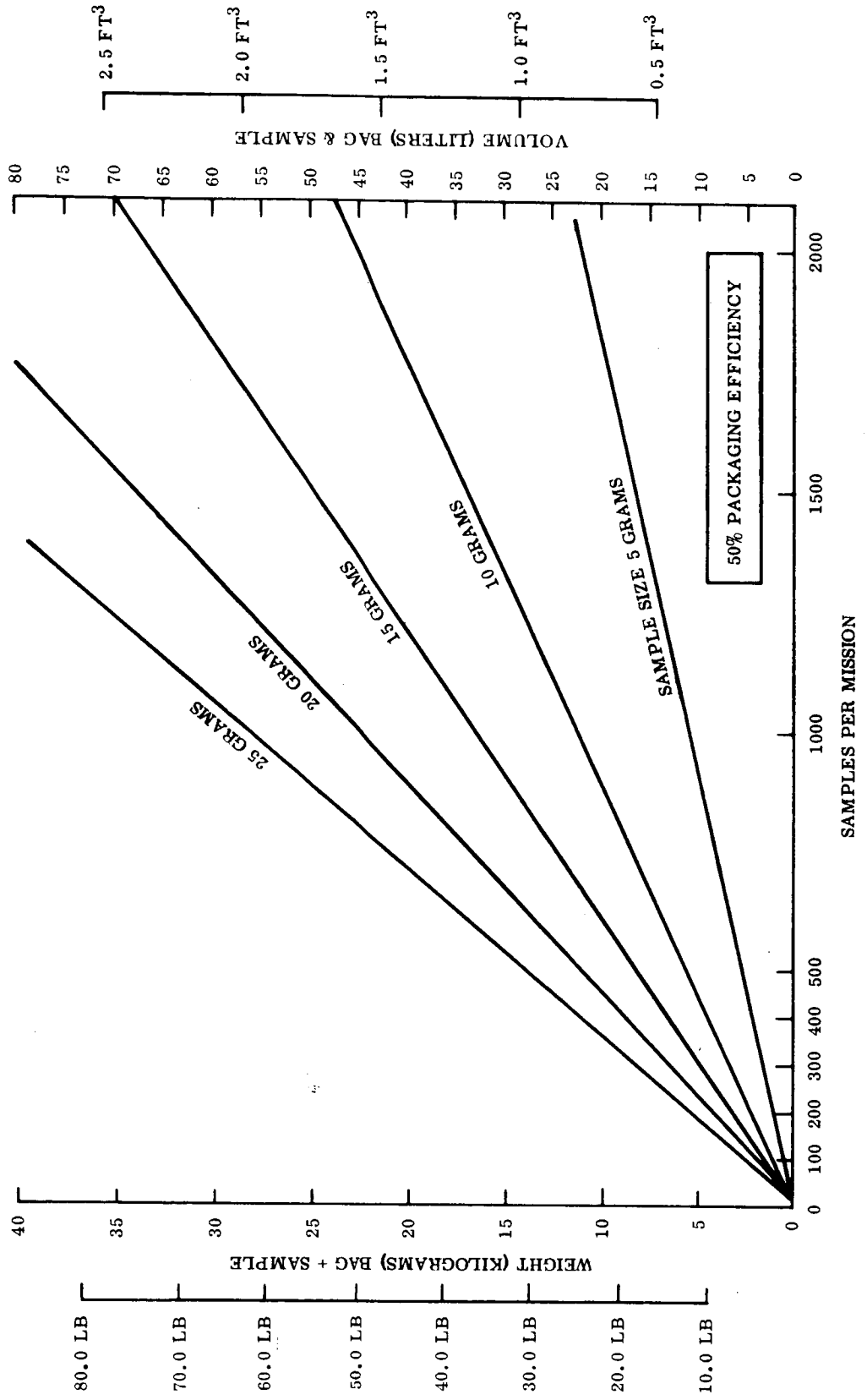


Figure 5-5. Final (Filled) Weight and Volume for Chemical Preservation

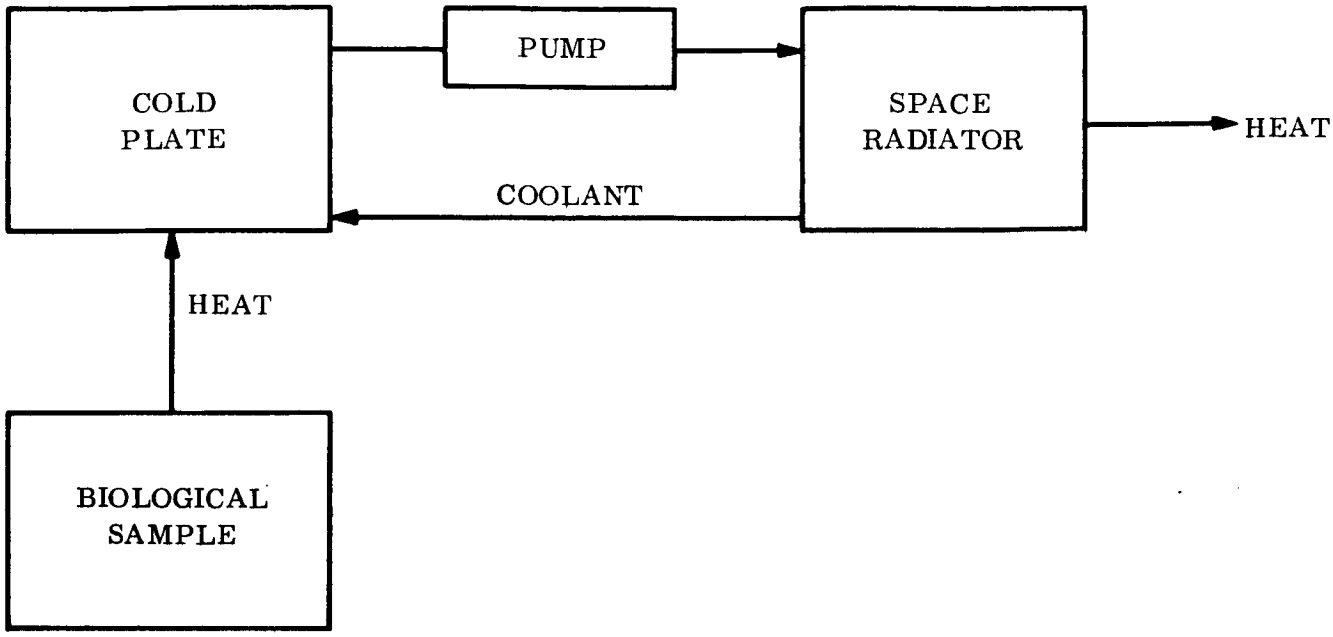


Figure 5-6. Refrigeration

Space Radiator is designed to the following characteristics:

Space Heat Sink Temperature:	$-100^{\circ}F$ ($-73.3^{\circ}C$)
Radiator Temperature:	$-60^{\circ}F$ ($-51.1^{\circ}C$)
Coolant Temperature:	$-10^{\circ}F$ at outlet ($-24.6^{\circ}C$)
Heat Rejection Rate:	10 watts/ft^2
Radiator Weight (includes coolant plumbing etc.)	1.0 lb/ft^2 or 0.1 lb/watt

The characteristics are graphically depicted in Figure 5-7.

Coolant Pump design is based on existing hardware. Assuming that the coolant has a $10^{\circ}F$ ($-13.5^{\circ}C$) drop through the storage box, and that the coolant has a specific heat of 0.5 BTU/lb$^{\circ}F$, then:

$$Q = W\ Cp\ \Delta\ t$$

$$Q\ (\text{watts}) \times 3.415\ \text{BTU/watt-hr} = W\ \text{lb/hr} \times 0.5\ \text{BTU/lb}^{\circ}\text{F} \times 10^{\circ}\text{F}$$

$$Q\ (\text{thermal watts}) = 1.47\ W\ (\text{lb/hr})$$

Assuming a standard coolant pressure drop through the system of 50 psi (115 ft of water) and an overall pump efficiency of 10 percent, than the pump electrical power requirement can be related to the thermal load Q.

$$P = \frac{\frac{Q}{1.47}\ (\text{lb/hr}) \times 115\ \text{ft}}{0.1 \times 2655\ \text{ft lb/hr}}$$

$$P\ (\text{electrical watts}) = 0.295Q\ (\text{thermal watts})$$

Figure 5-8 graphically illustrates the pump power requirement. The weight of the pump will not change significantly from a 2.0 pound estimate for all thermal loads.

Sample Storage Box. The design of the sample storage box is based on one main factor. The initial chilling of the sample will be negligible compared to the overall thermal losses of the box. This is seen to be true by an example:

Consider a 10 gram sample which must be chilled from 100°F (37.8°C) to 40°F (4.4°C) in five minutes, assuming a conservative specific heat of 1.0 BTU/lb$^{\circ}$F.

$$Q = W\ Cp\ \Delta\ t = \frac{10\ \text{gm}}{454\ \text{gm/lb}} \times \frac{1.0\ \text{BTU}}{\text{lb}^{\circ}\text{F}} \times \frac{60^{\circ}\text{F}}{1} \times \frac{60\ \text{min/hr}}{5\ \text{min}}$$

$Q = 1.6$ BTU/hr or 0.47 watts cooling required.

The heat leakage into the enclosure (box) is calculated as follows: The insulation is assumed

to be polyurethane foam* with a thermal conductivity, K, of 0.2 BTU-in/hr $ft^{2o}F$ (Data from Nopco Chemical Co.). Assuming that the insulation is two inches thick and that edge losses and door openings will increase the losses by a factor of two, then the overall heat loss is 0.2 BTU/hr $ft^{2o}F$. The area is determined by assuming the enclosure is cubical in shape so that the cold surface area is $A = 6X^2$, where X = (volume) 2/3. Also assuming that the samples have a specific gravity of 1 and are packaged with 50 percent efficiency, then the surface area $A = 6(2 \times \text{sample weight})^{2/3}$. The overall heat leakage is then:

$$Q = K A \Delta t$$

$$Q \text{ watts} = \frac{0.2 \text{ BTU/hr ft}^{2\,o}\text{F}}{3.415 \text{ BTU/watt hr}} \times \frac{6\,(2 \text{ W cm}^3)^{2/3}}{930 \text{ cm}^2/\text{ft}^2} \times \Delta t$$

$$Q = 0.0006\,(W)^{2/3}\,\Delta t \text{ where } W = \text{grams}$$

For example, the box for 1000 - 10 gram samples at 35^oF with a 75^oF ambient will have a heat leakage of 11.1 watts. From Figures 5-7 and 5-8, the coolant pump will require approximately 3 watts and the radiator size will be approximately 1.2 ft^2 and weight 1.2 pounds.

The storage box construction is polyurethane foam sandwiched between aluminum or plastic sheets for strength. The box contains shelves for storage of the samples. The shelves are constructed with the coolant flowing through alternate levels with spring loaded shelves on the other levels. The spring loaded shelves hold the samples against the cooled shelves in the zero gravity environment. A pallet may be used to hold groups of samples in place and to permit easy storage and removal. See Figure 5-9. The storage box has a hinged door and a non-reactant opening device for the zero gravity environment. Boxes of a similar design (4) with two cubic feet (56,00 cm^3) internal volume weighed approximately 25 pounds. Assuming an approximely linear scaling factor of one pound per 2000 cm^3 of storage volume,

*Self-extinguishing foams are used for fire prevention. Other insulations such as GE P-Zero are also available which use an evacuated jacket and non-flammable materials (aluminum and fiberglass). These are better insulations but are more costly and weigh more.

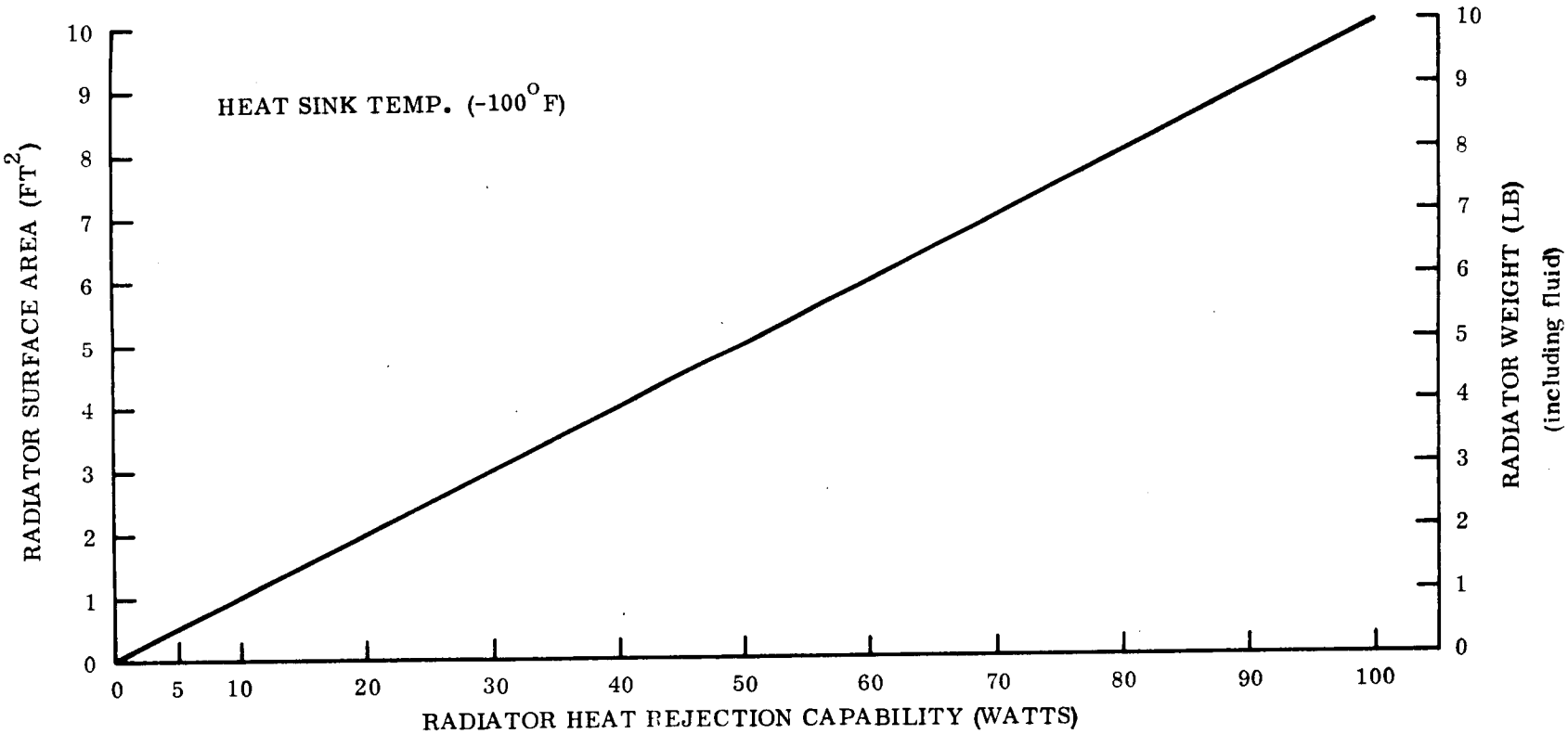


Figure 5-7. Space Radiator Characteristics

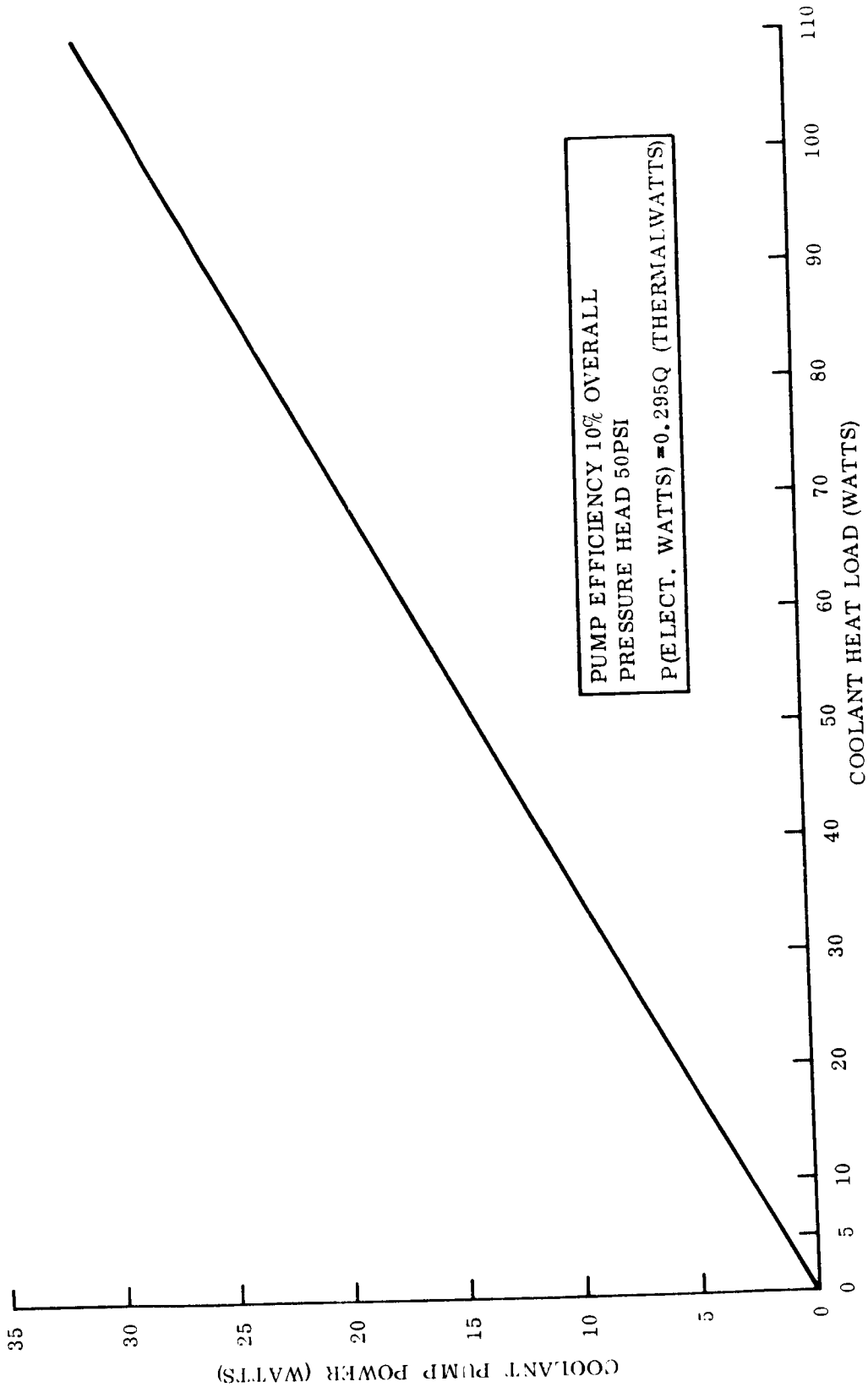


Figure 5-8. Coolant Pump Characteristics

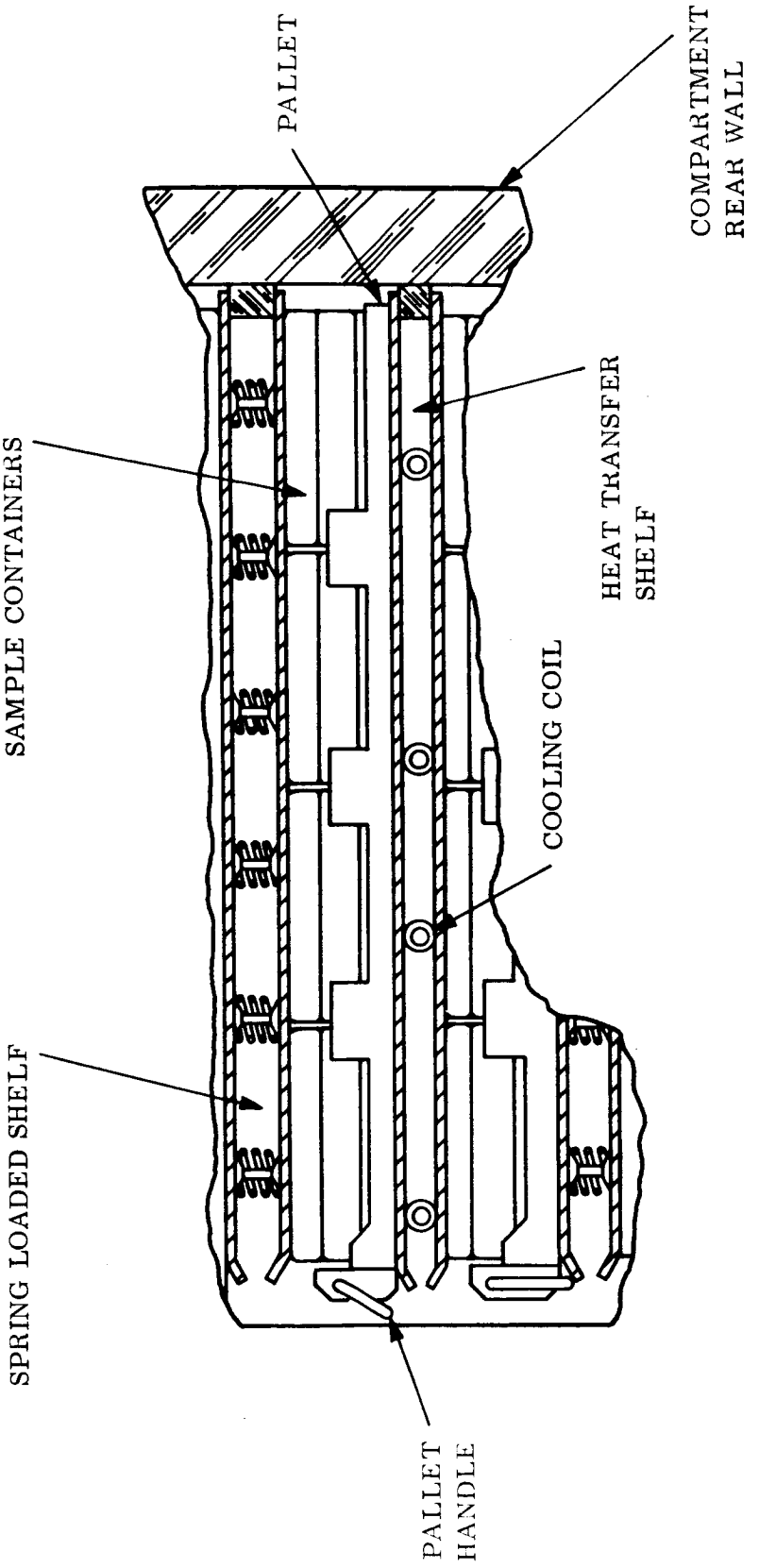


Figure 5-9. Restraint Mechanism and Shelf Arrangement

including storage bags, then the weight of the box for various amounts of samples can be determined. Total box volume will be approximately four times the sample volume, including a 50 percent sample packaging efficiency. See Figure 5-10.

Combining Figures 5-7, 5-8 and 5-10, the overall weight, volume and power requirement for refrigeration preservation is shown in Figures 5-11, 5-12 and 5-13.

5.3.3 FREEZING

Firm data on the thermodynamic properties of biological samples was not found in the literature; consequently, estimates were made based on the amount of salt commonly found in the sample fluids.

To completely solidify samples of blood, plasma or urine, a temperature of -30°C (-22°F) will be required, as the solidification of the NaCl-water eutectic does not occur until -28°C (-18.4°F). Temperatures above this point will only partially freeze a sample; while ice crystals will separate out, the liquid phase will become more concentrated until the autectic composition is reached. From this point on, the liquid will be converted to a solid mixture of ice and salt crystals only by going to a temperature below the eutectic temperature. This is admittedly a simplification, since the samples will consist of complex mixtures of water, salts, and organic matter; however, by going to a temperature slightly below the NaCl-water eutectic temperature any additional liquid phases should be frozen out. By converting all liquid phases to solid, the migration of reactants is halted which will prevent reactions, except those of the simple breakdown of molecules, from occurring. It may be necessary to avoid damage to biologically active compounds in freezing by preserving one portion of a sample by refrigeration and a second portion by freezing.

Rapid freezing may be accomplished by retaining the sample in a metal sponge with a large surface area and good heat transfer to the cooling surface. Commercial thin film freezing techniques may also be applicable. For example, a method of increasing the cooling area and thus decreasing the freezing time is to utilize a "cold finger" design. This consists of a

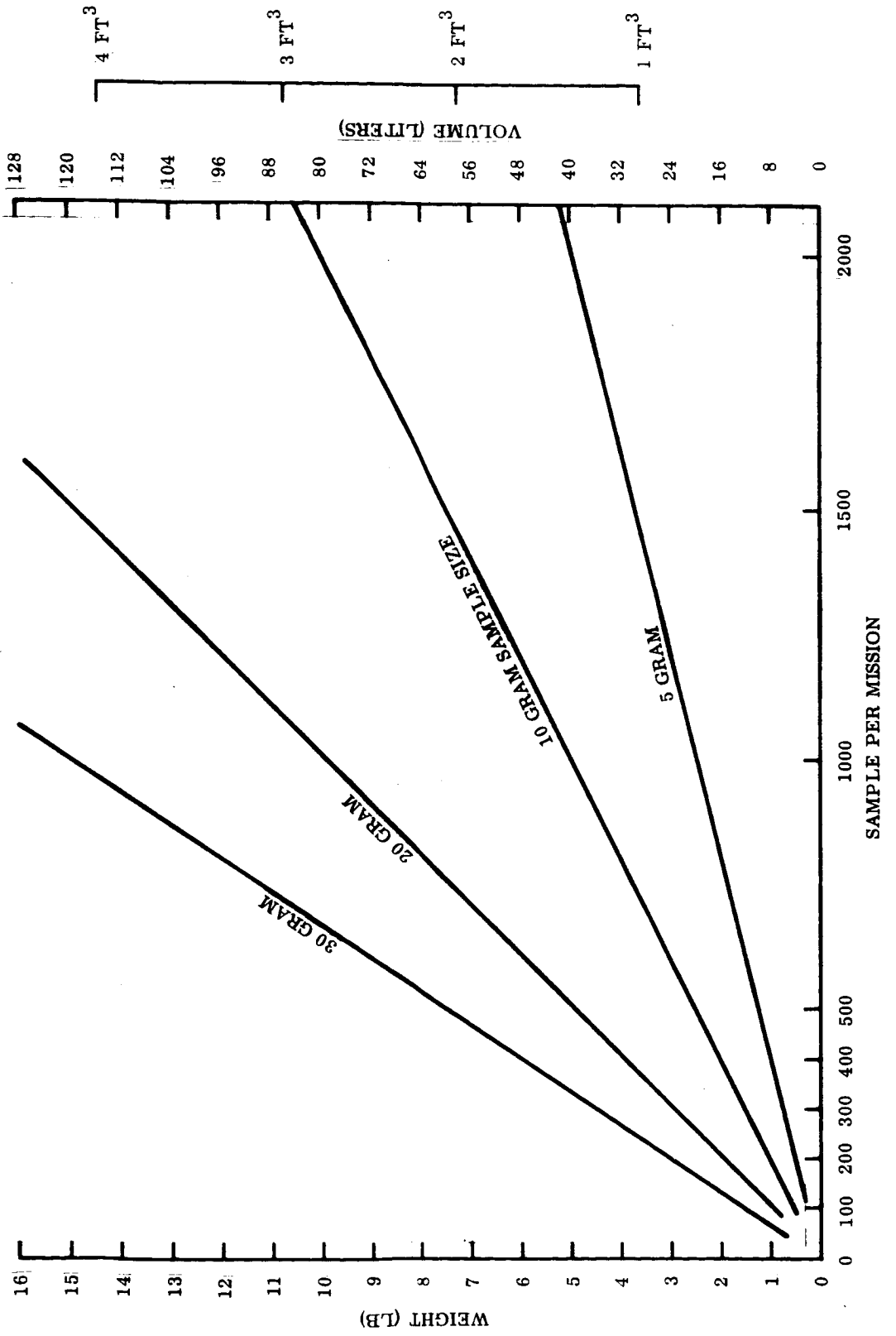


Figure 5-10. Refrigerated Sample Storage Box

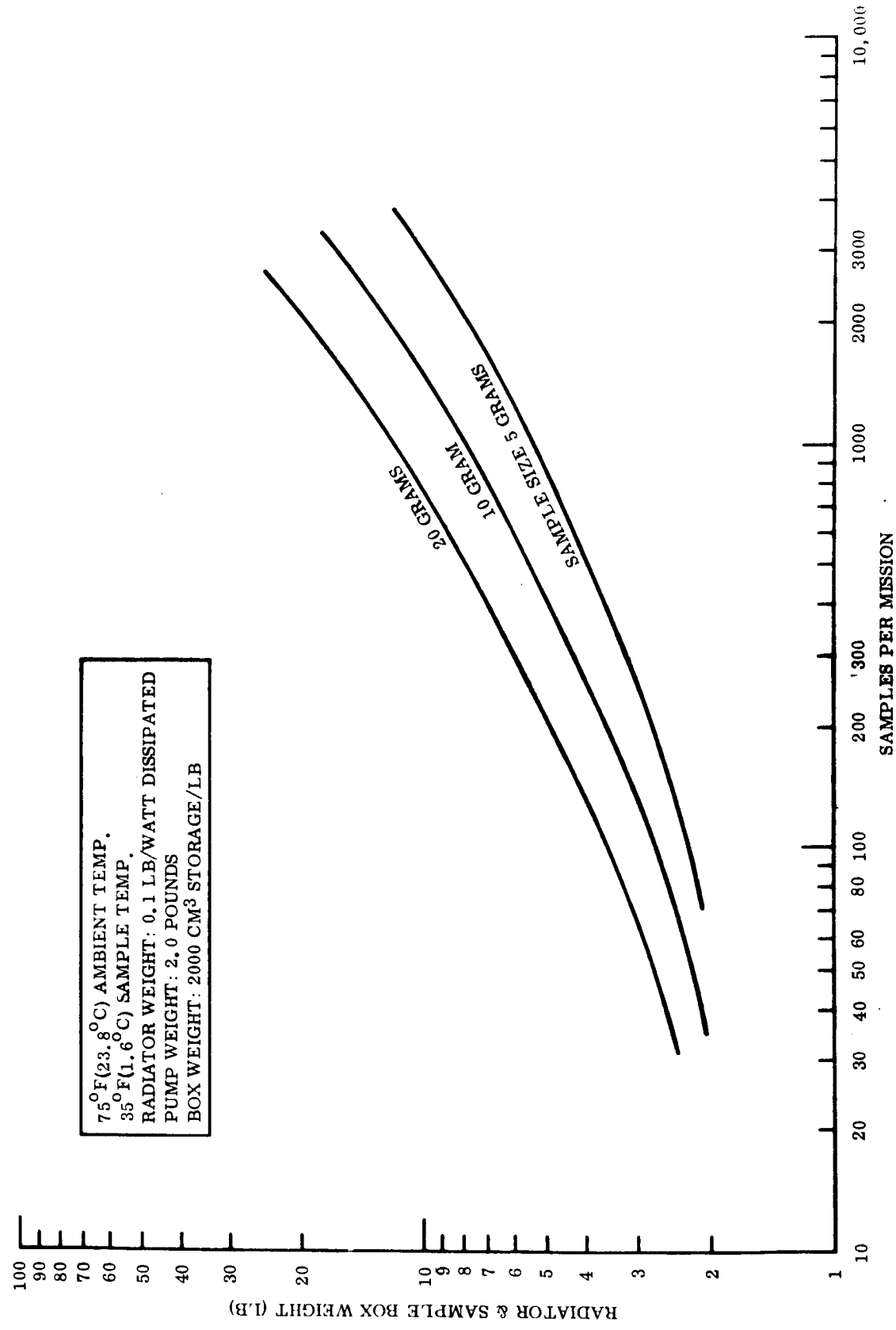


Figure 5-11. Preservation by Refrigeration - Weight

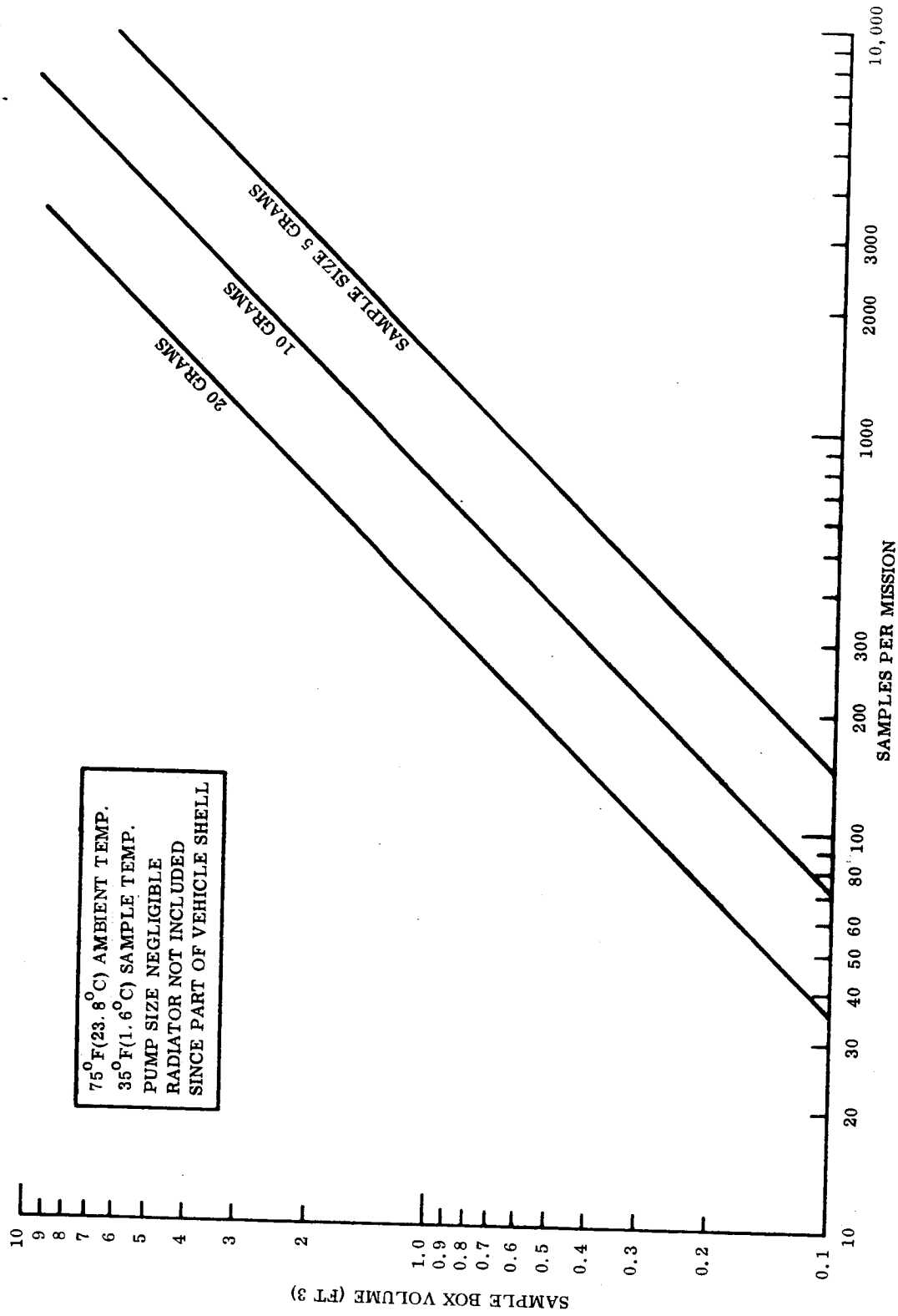


Figure 5-12. Preservation by Refrigeration - Volume

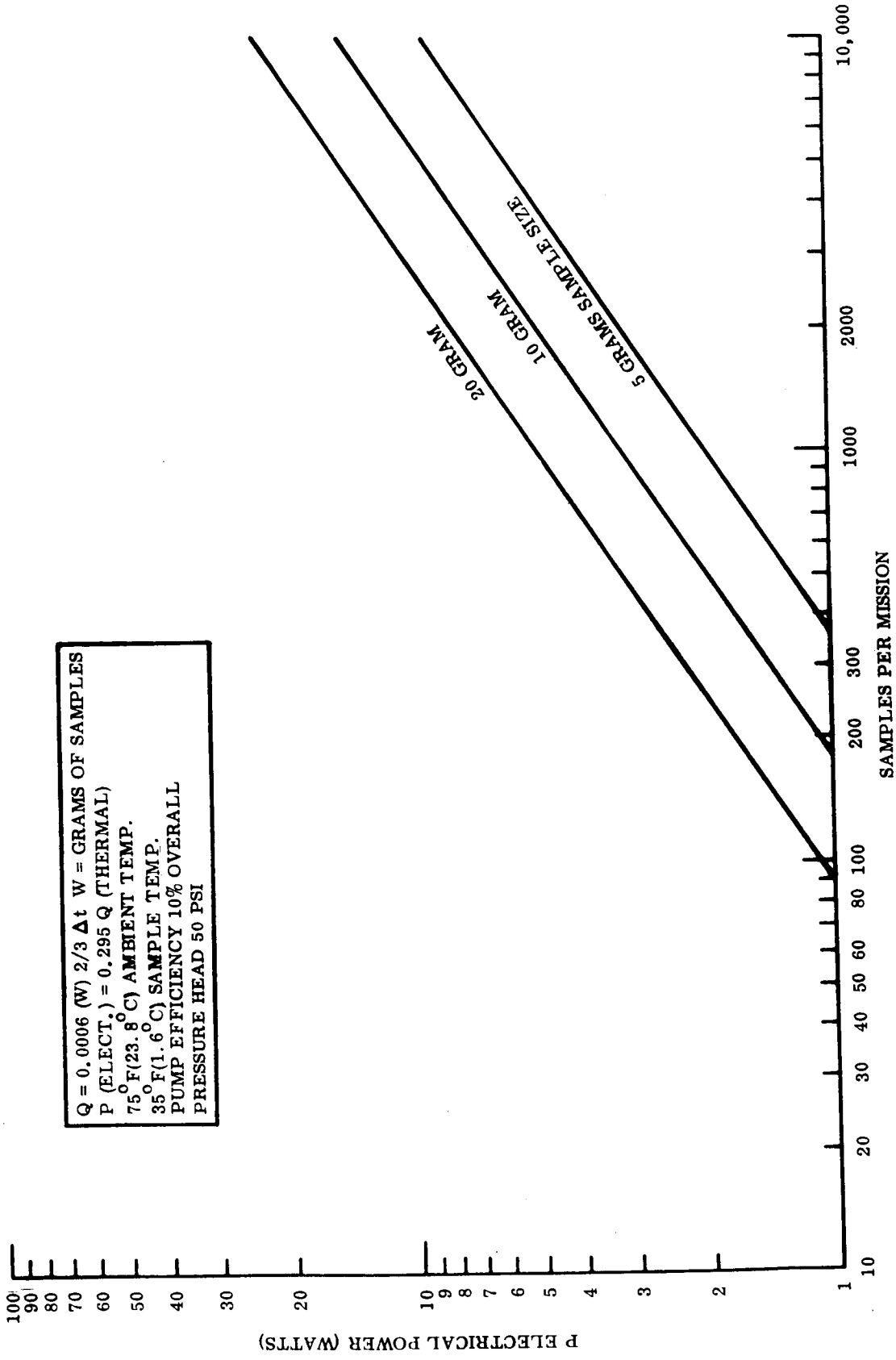


Figure 5-13. Preservation by Refrigeration - Power Requirement

fairly large cooled cylindrical shape placed at the center of the sample. See Figure 5-14. The sample is spread in a thin layer over the large area of the "cold finger" and is rapidly frozen.

The Freon vapor cycle cooling unit is the selected approach to preservation by freezing at temperatures near $-40^{\circ}F$. Assuming that the heat from the cooling unit is rejected to the vehicle environmental control system coolant loop at $60^{\circ}F$ ($15.4^{\circ}C$), the theoretical coefficients of performance (COP) of the unit is calculated for various operational temperatures. See Figure 5-15.

$$\text{(Carnot Cycle) COP} = \frac{T_1}{T_2 - T_1}$$

T_1 = absolute operating temperature

T_2 = absolute ambient temperature ($60^{\circ}F$) = $520^{\circ}R$

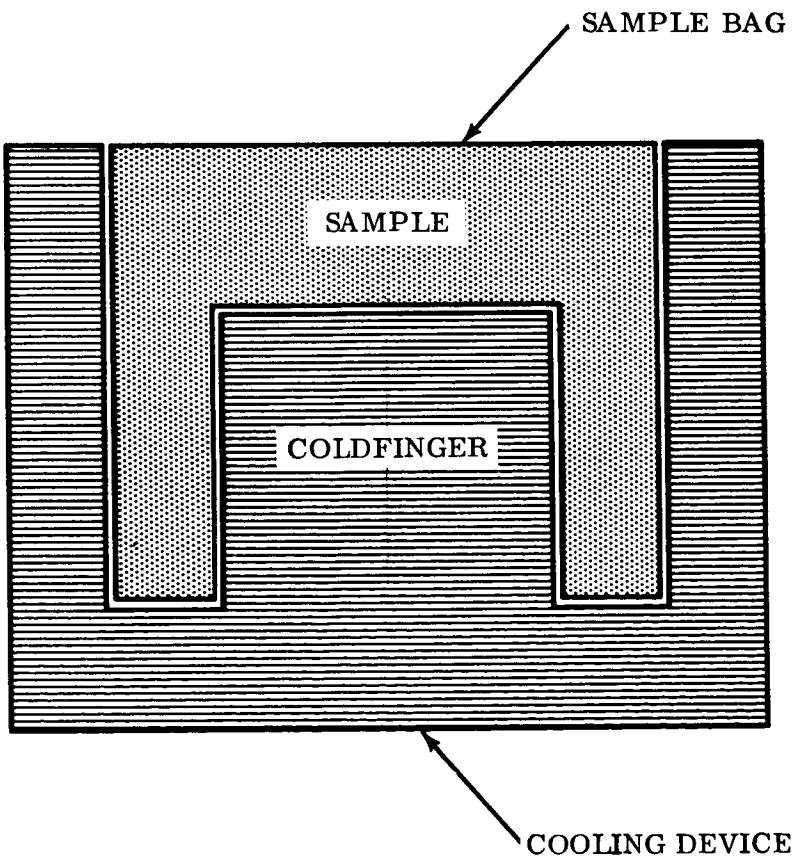


Figure 5-14. "Coldfinger" Sample Freezing Arrangement

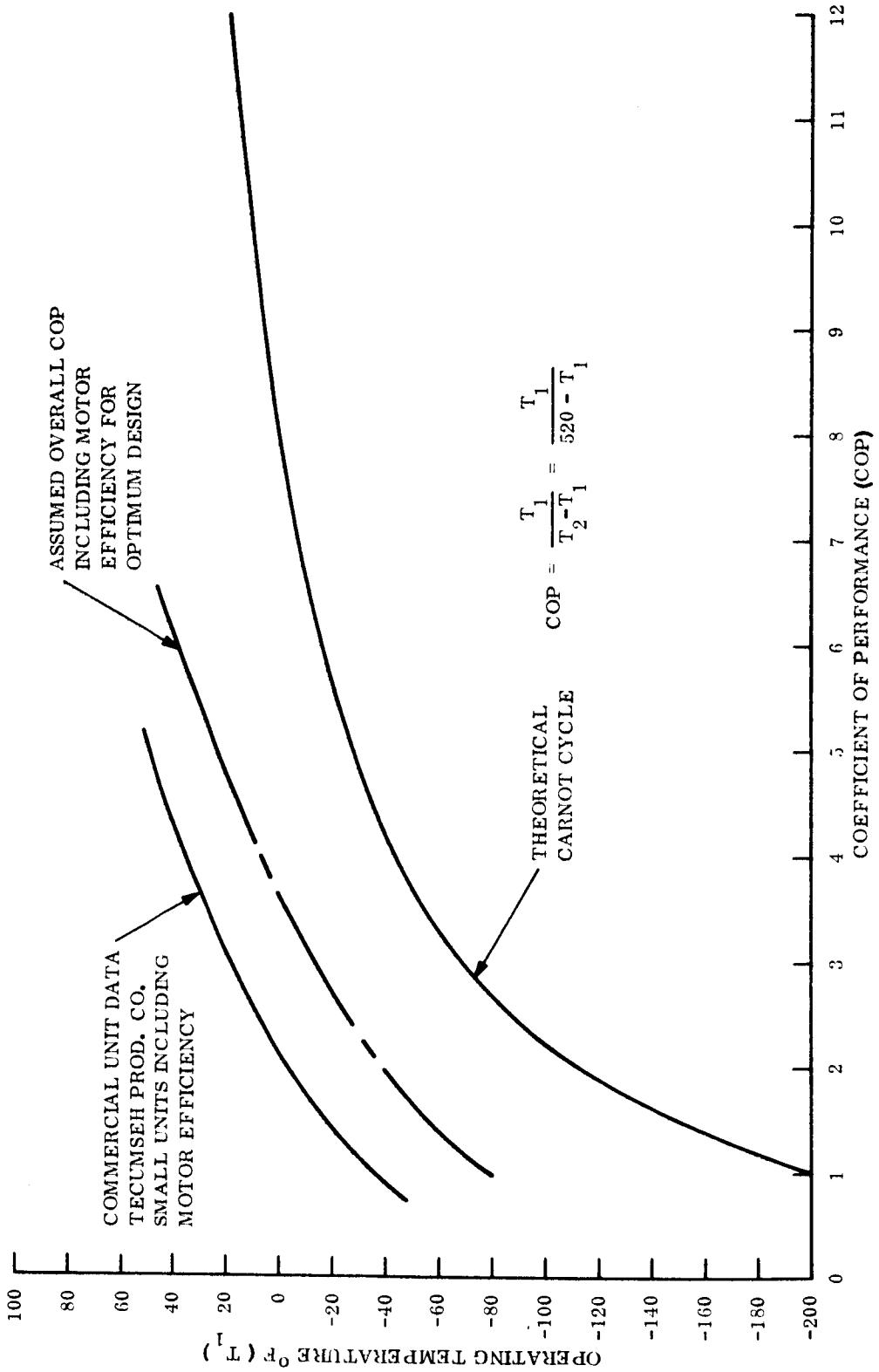


Figure 5-15. Freon Vapor Cycle COP

Also shown in Figure 5-15 are the overall COP's of Commercial units including the motor efficiency and an assumed overall COP including the motor, which could be expected from an optimized design specifically for this application. From the above assumptions, the electrical power requirement is accurately calculated and shown in Figure 5-16.

The weight and physical size of Freon vapor cycle units are governed by practical considerations such as motor and compressor sizes, packaging, etc. Available commercial and a few aircraft unit data were used to establish the weight and package size curves shown in Figures 5-17 and 5-18.

Note that the commercial units establish an upper limit and help to determine slopes of the aircraft data lines.

The design of the freezing unit is based on three factors:

1. The unit is designed to handle the end number of samples.
2. The Freon vapor cycle unit will be greatly overdesigned for the initial number of samples, but is designed for the ultimate load.
3. There will be enough thermal mass in the sample storage enclosure and previous samples to absorb the thermal load of the final samples without a significant load on the Freon vapor cycle unit.

Therefore, the Freon vapor cycle unit is designed to handle only the heat leakage into the sample storage enclosure. The heat leakage into the enclosure is calculated as follows: The insulation is assumed to be polyurethane foam with a thermal conductivity K of 0.2 BTU-in/hr ft^2 ^oF (data from Nopco Chemical Co.). Insulation one order of magnitude better are available but they weigh more and require special design procedures (4). Assuming that the insulation is two inches thick and that edge losses and door openings will increase the losses by a factor of three, than the overall heat loss is 0.3 BTU/hr ft^2 ^oF. The area is determined by assuming that the sample enclosure is cubical in shape so that the cold surface area $A = 6X^2$ where $X = (\text{volume})^{1/3}$. Therefore, $A = 6\ (\text{volume})^{2/3}$.

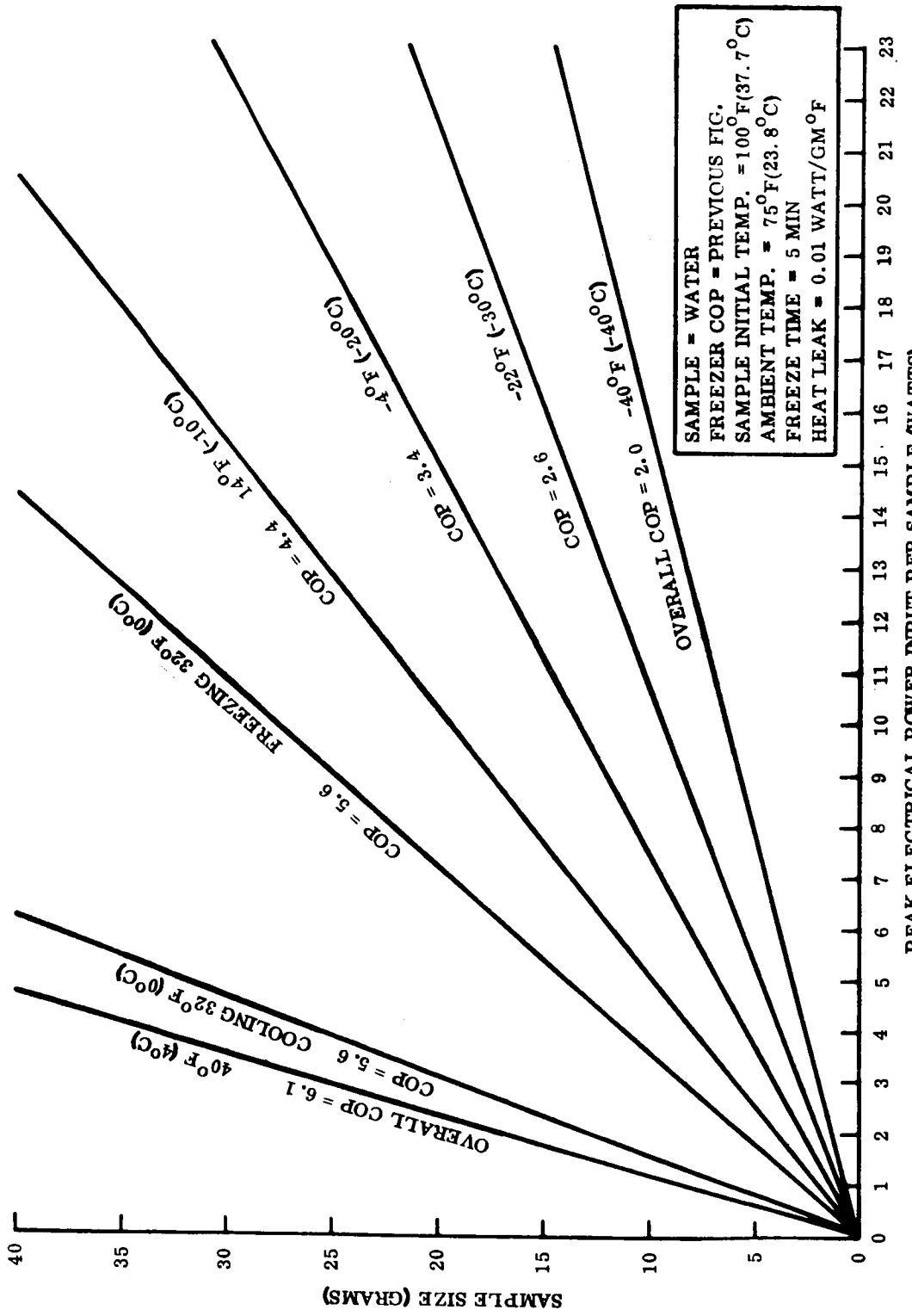


Figure 5-16. Sample Size versus Power Input - Freon Vapor Cycle Unit

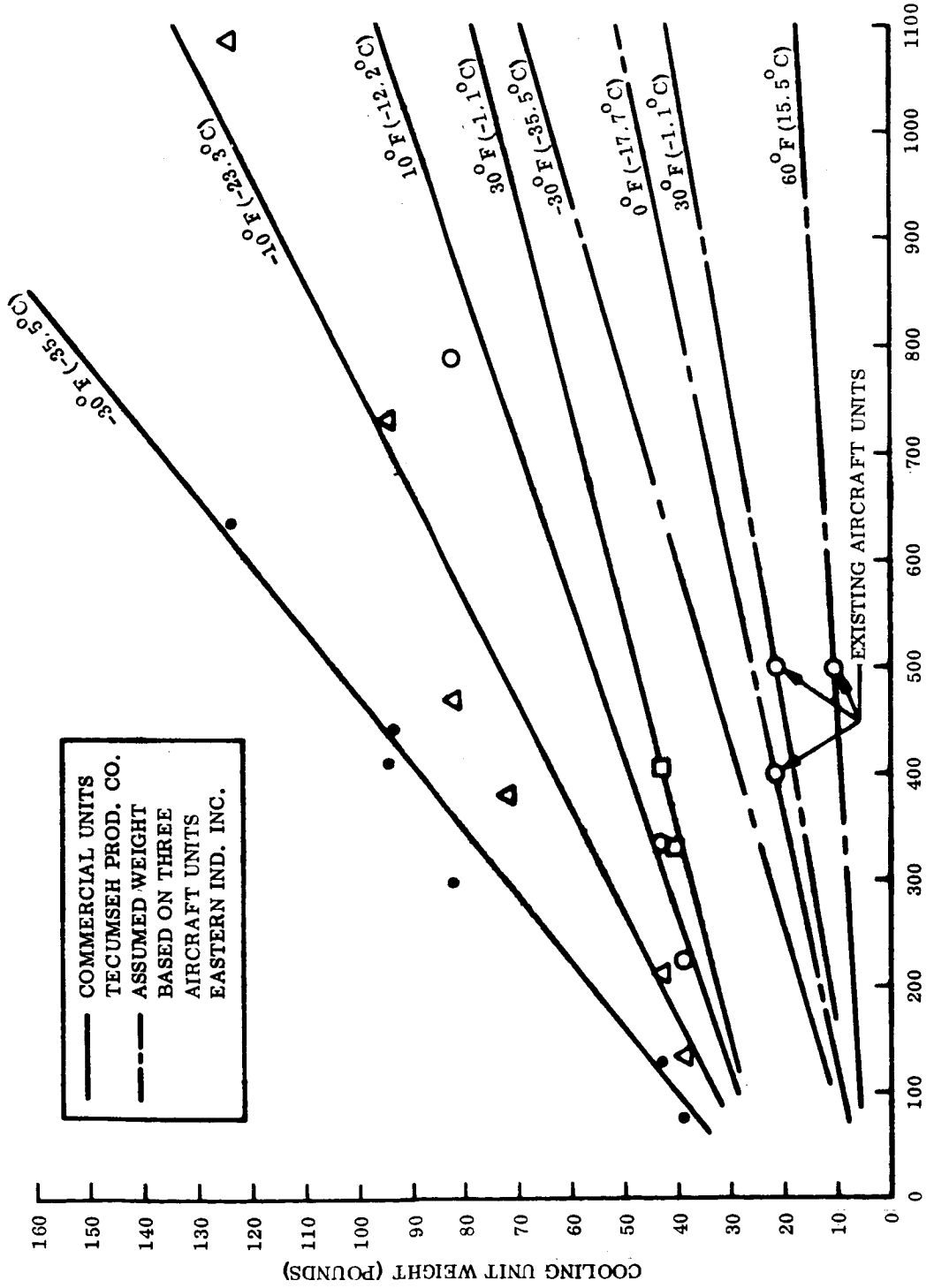


Figure 5-17. Freon Vapor Cycle Unit Weight versus Cooling Load

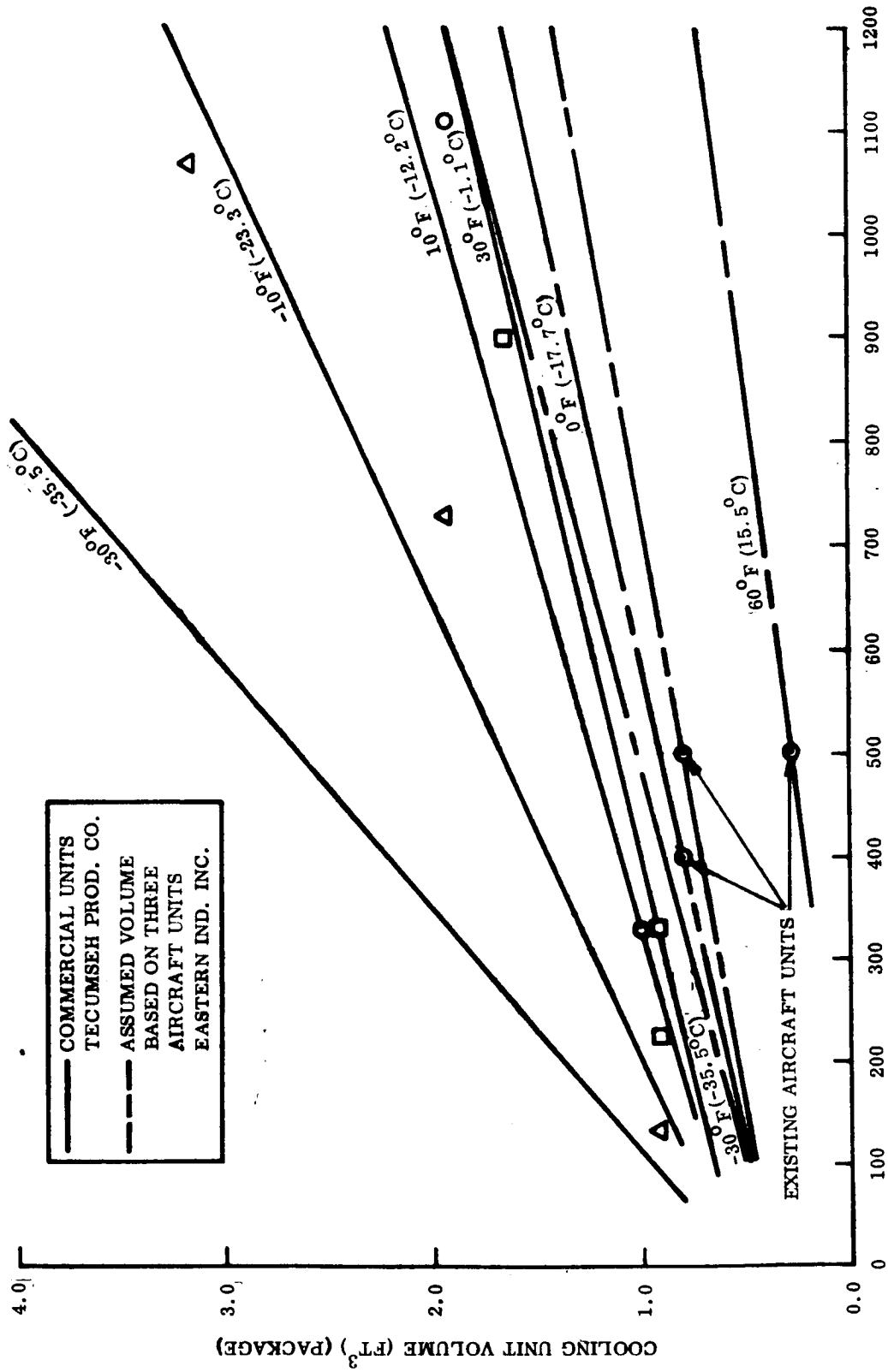


Figure 5-18. Freon Vapor Cycle Unit Volume versus Cooling Load

Also assuming that the samples have a specific gravity of one and are packaged with 50 percent efficiency, then the surface area $A = 6\ (2 \times \text{sample weight})^{2/3}$. For example, 100-10 gram samples would have a surface area of $A = 6(2 \times 100 \times 10)^{2/3} = 955\ cm^2$. The overall heat leakage is then:

$$Q = K\ A\ \Delta\ t$$

$$Q\ \text{watts} = \frac{K\ 0.3\ \text{BTU/ hr ft}^2\ ^{o}\text{F}}{3.415\ \text{BTU/watt hr}} \times \frac{6\ (2W\ \text{cm}^3)^{2/3}}{930\ \text{cm}^2/\text{ft}^2} \times \Delta\ t\ ^{o}\text{F}$$

$$Q\ \text{watts} = 0.0009\ (W)^{2/3} \times \Delta\ t \quad \text{where } W = \text{grams}$$

The electrical power requirement for the refrigeration and/or freezing unit is then:

$$\text{Power (watt)} = \frac{0.0009\ (W)^{2/3}\ \Delta\ t}{COP} \qquad W = \text{grams}$$

For example: 1000-10 gram samples at $-40^{o}F$ ($-40^{o}C$) with an ambient of $75^{o}F$ ($23.8^{o}C$) and a COP of 2.0 from Figure 5-15, the electrical power required is:

$$\text{Input Power} = \frac{0.0009}{2}\ (10,000)^{2/3} \times 115 = 24\ \text{watts}$$

The curves shown in Figures 5-19, 5-20 and 5-21 are determined in a similar manner for various sample size and temperatures. Once the power and cooling requirements are determined, then the weight and volume of the Freon vapor cycle unit is determined and illustrated in Figures 5-22 and 5-23. For example, from these figures, 1000-10 gram samples stored at $-40^{o}F$ ($-40^{o}C$) will require a cooling unit which weighs eight pounds and has a volume of 0.48 cubic feet.

The sample storage module (box) is basically the same as described in the refrigeration section. The box weight is approximately one pound for every 2000 cm^3 of storage volume and the total box volume is approximately four times the sample volume, including packaging efficiency.

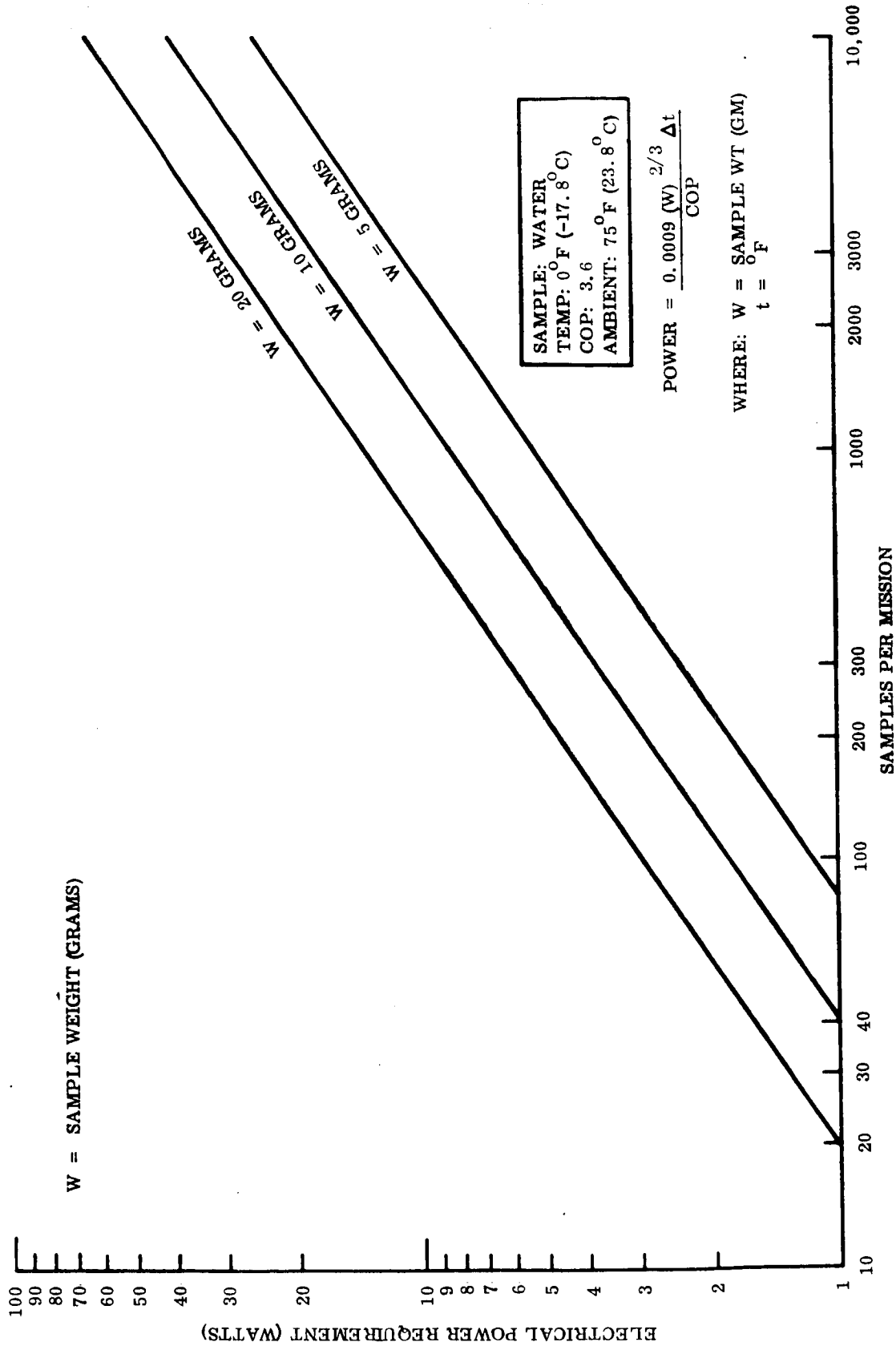


Figure 5-19. Power Requirement - Freon Vapor Cycle Unit at 0°F

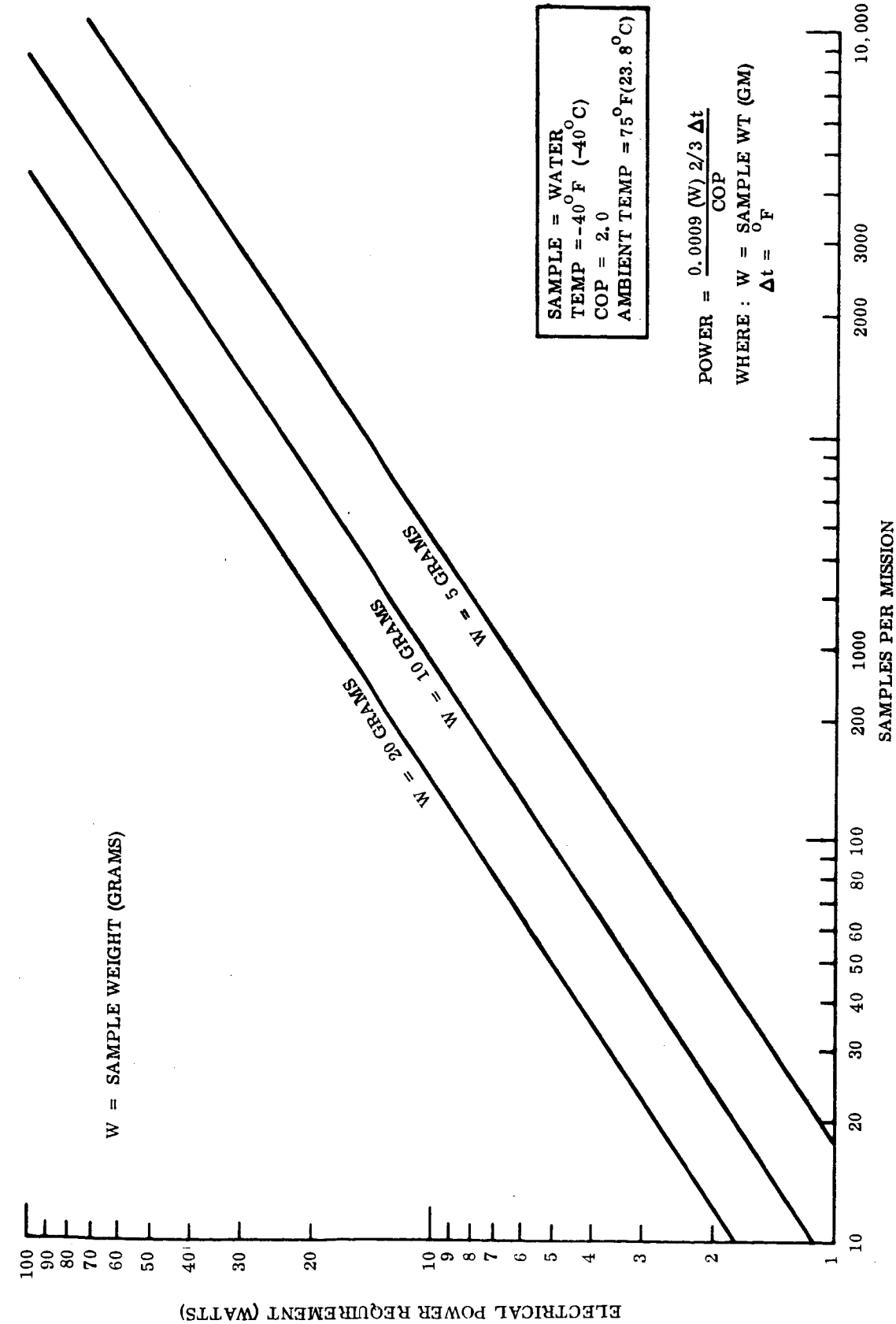


Figure 5-20. Power Requirement - Freon Vapor Cycle Unit at -40°F

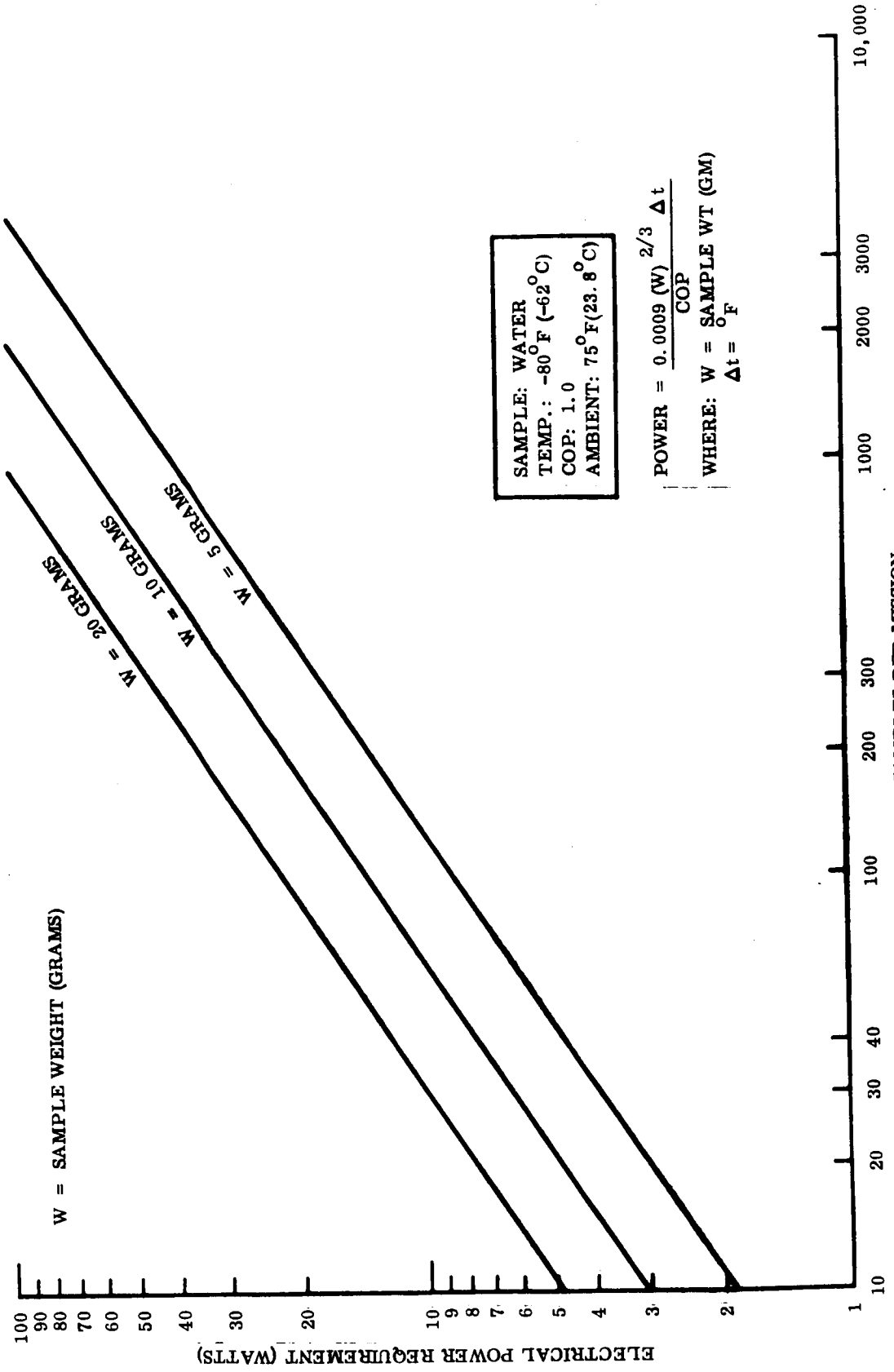


Figure 5-21. Power Requirement - Freon Vapor Cycle Unit at -80°F

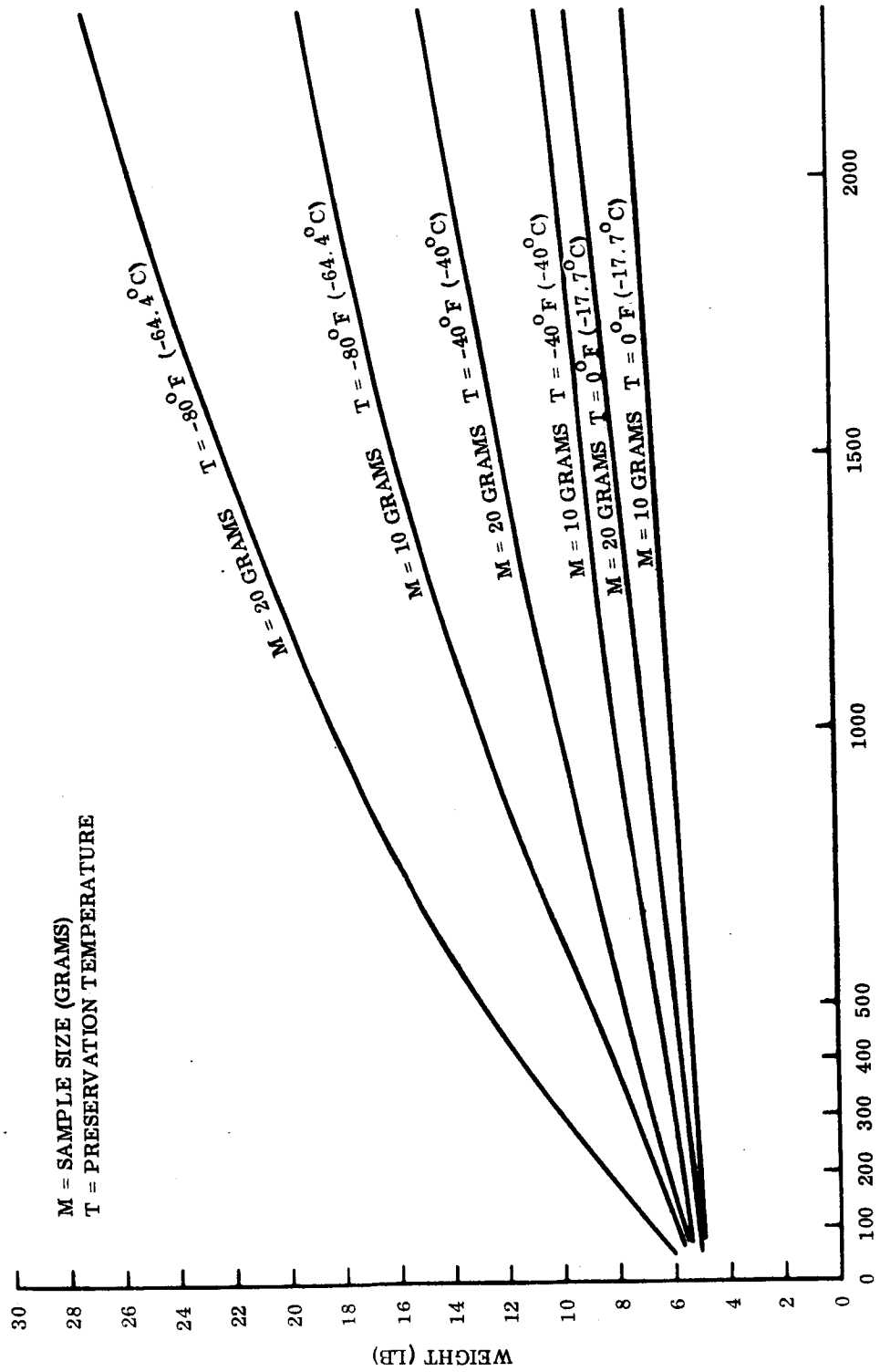


Figure 5-22. Preservation by Freon Vapor Cycle Unit - Weight

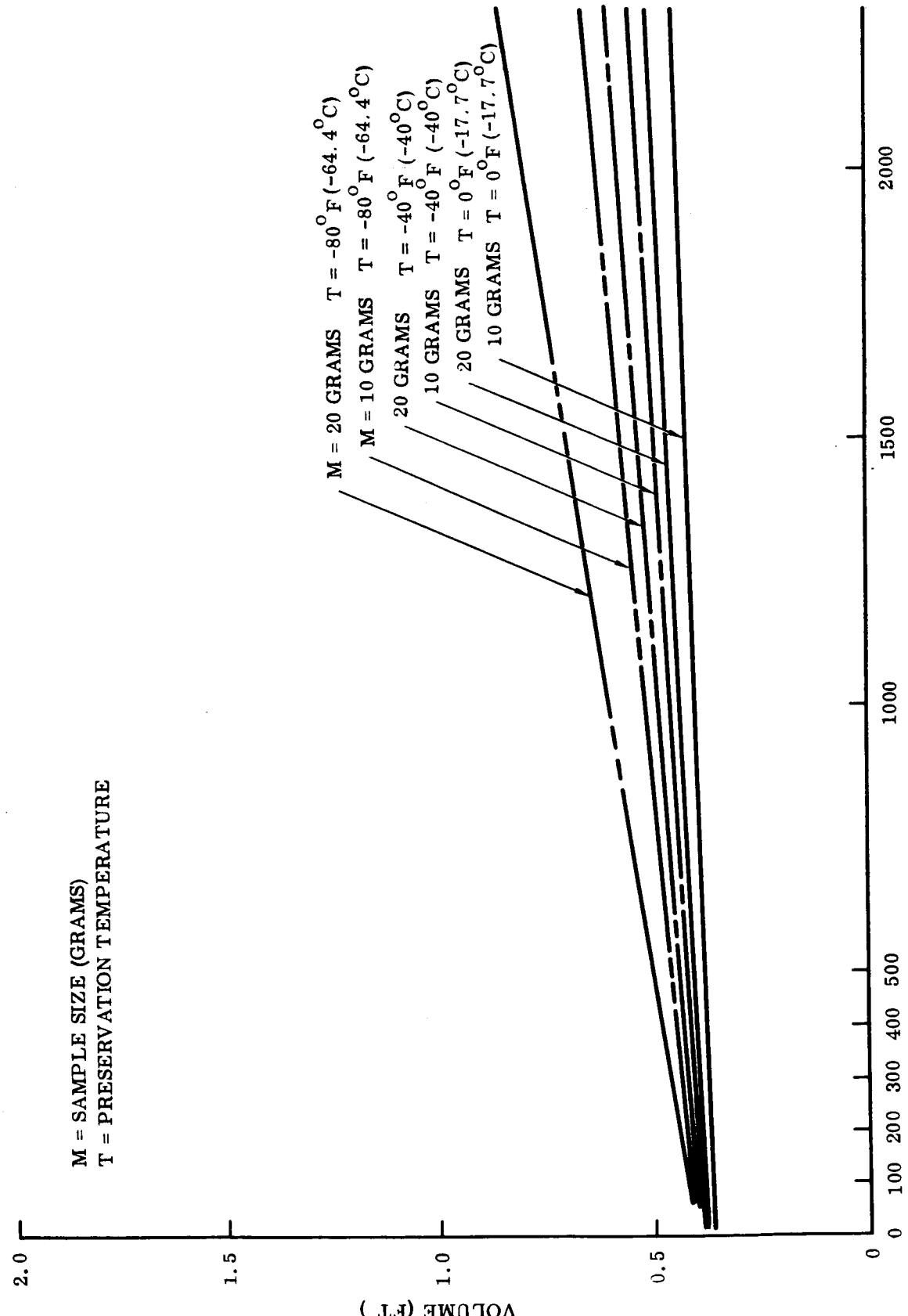


Figure 5-23. Preservation by Freon Vapoe Cycle Unit - Volume

The total system weight and volume are thus determined by combining the individual characteristics of the Freon vapor cycle unit and the storage box. See Figure 5-24, 5-25 and 5-26.

No Freon vapor cycle unit is presently known to be available for space flight operation. The major problem with space flight is the zero gravity environment, which affects the heat transfer as well as the lubrication of the unit. Zero gravity tests in airplanes at Wright-Patterson Air Force Base and drop towers at NASA-Lewis Research Center have shown that vapor cycle units are feasible for space application. Further development is now required.

5.3.4 VACUUM DISTILLATION

The selected approach to preservation by vacuum distillation is to retain the sample in a porous material and subject it to space vacuum. Gas entrainment in the sample will cause violent boiling if initially exposed to a low pressure; therefore, the pressure is lowered in two stages. The first stage lowers the pressure to approximately 10.0 mm Hg, where the sample is degassed, The second stage lowers the pressure for complete distillation. All controls are manual so no electrical power is required. If the porous material is a metal with good heat conduction to the ambient environment, the vacuum distillation process will be quite rapid. A poor conducting material such as porous Teflon may act as a modulator of the boiling process, but this may also cause the sample to freeze as more heat is removed by boiling then is conducted to the sample. Preliminary trade-off studies and experimentation have been conducted to determine an optimum rate of boiling and size and configuration of the sample (aliquot) container; however, additional effort is required in this area.

Experimental tests utilizing vacuum distillation were conducted during a GE funded study. The variable test parameters were the container surface temperature, vacuum vent size, sample size, foam and copper wool inserts, repeated injection into sample container, metal containers and container configurations (25). Based on these tests, (typical data is shown in Figure 5-27) it will require approximately 10 minutes per gram of sample to achieve approximately 100 percent evaporation of the water in the sample; therefore, a large number

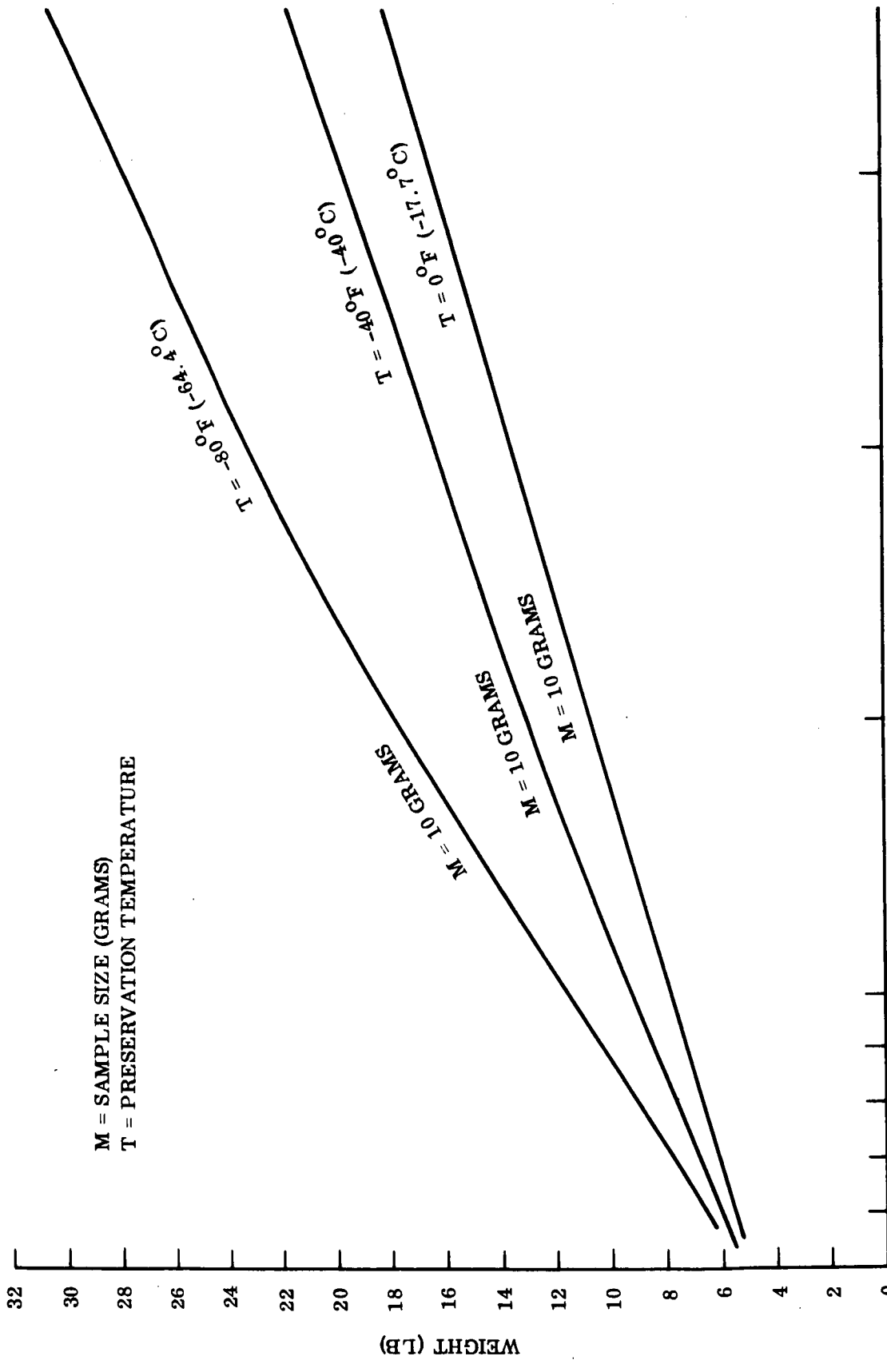


Figure 5-24. Preservation by Freezing - Weight

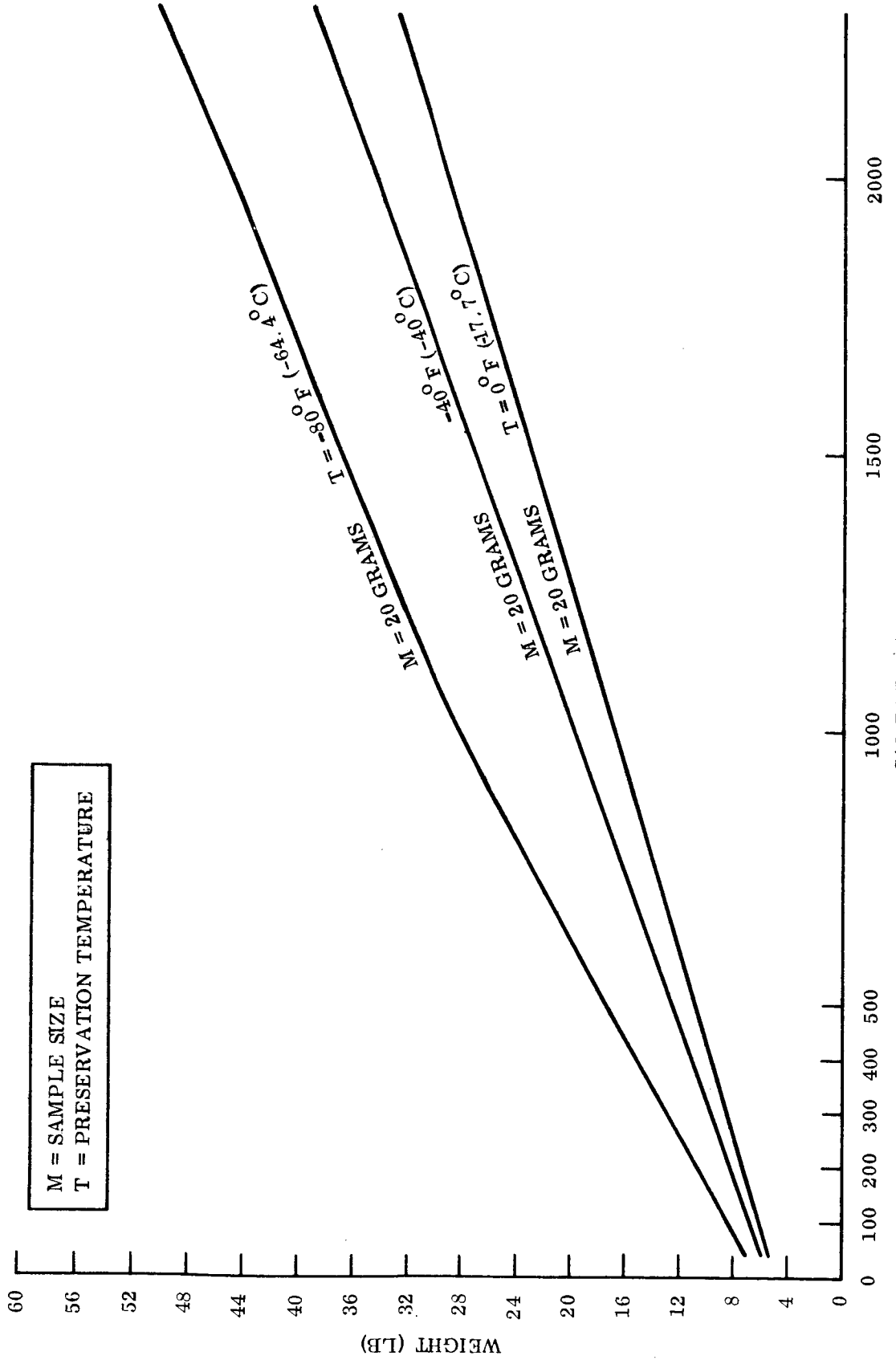


Figure 5-25. Preservation by Freezing - Weight

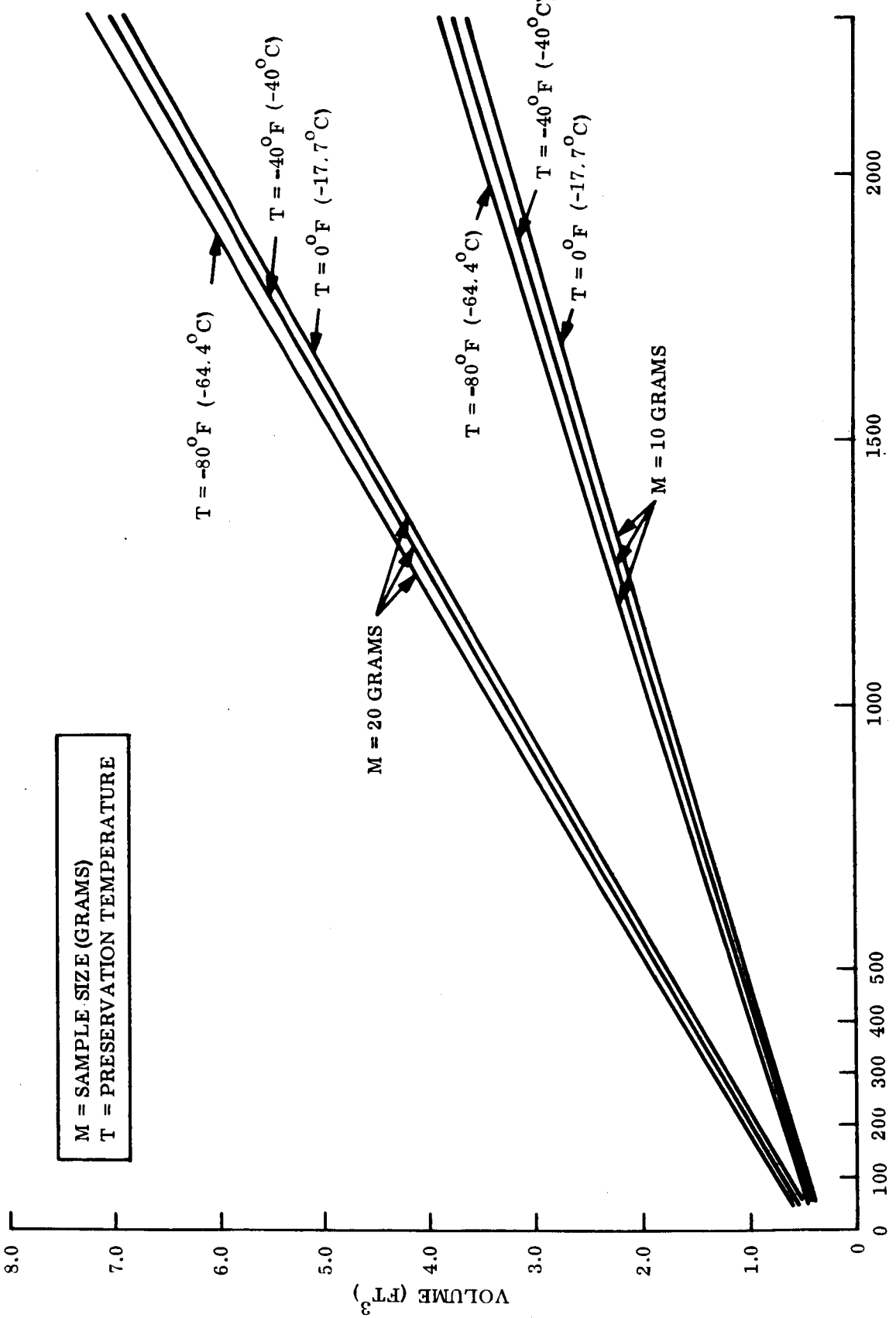


Figure 5-26. Preservation by Freezing - Volume

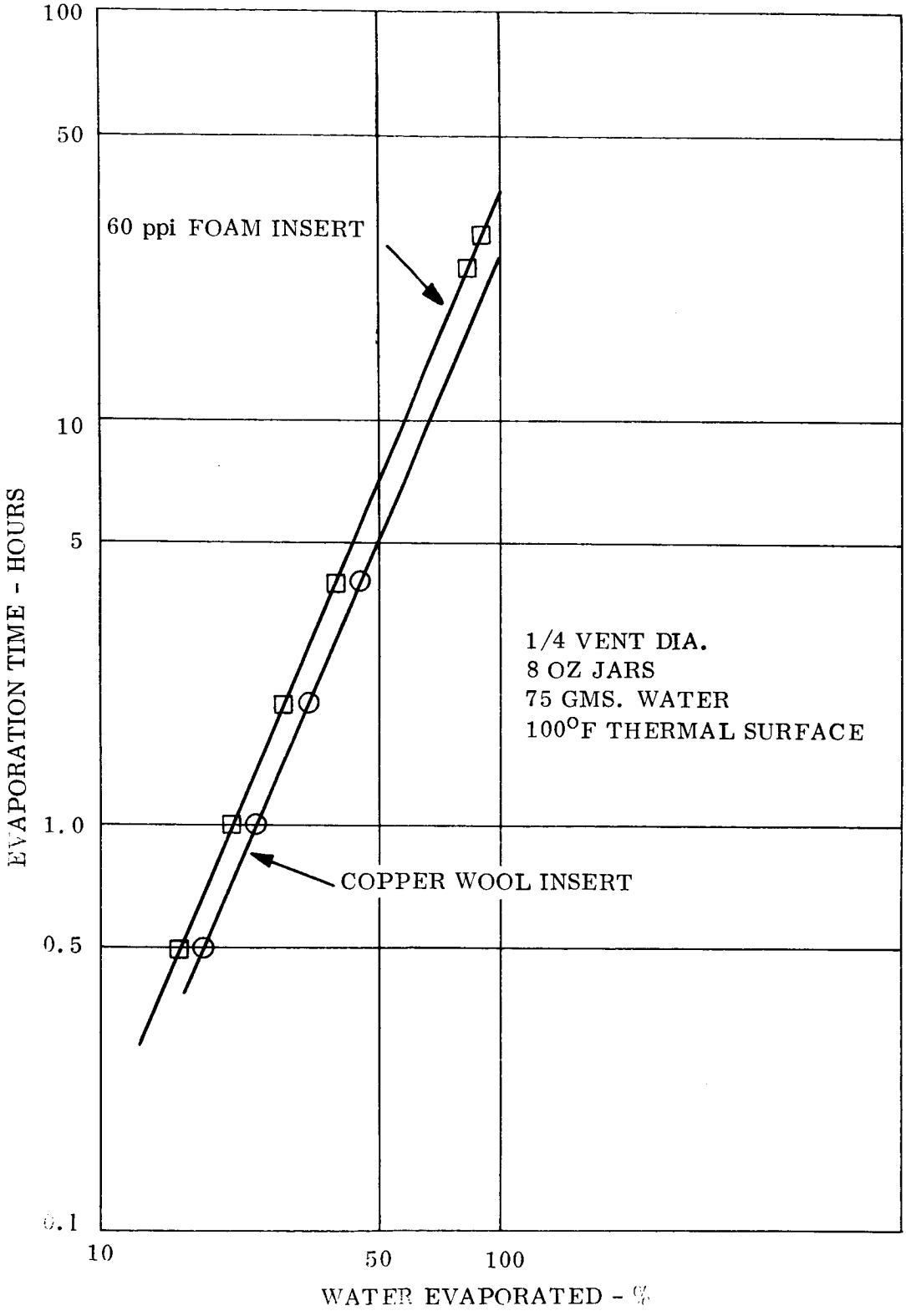


Figure 5-27. Typical Vacuum Distillation Test Data In Study of Effect of Metallic Insert

of drying chambers will be required if numerous samples are taken. Assuming that the system is utilized 25 percent of mission time, then the number of drying chamber required is calculated by:

$N = 0.000618\ MS$

N = No. of drying chambers
M= Weight of sample (grams)
S = No. of samples per mission

So that the 1000-10 gram samples, seven drying chambers are required.

$$N = 0.000618 \times 10 \times 1000 = 6.18$$

A conceptual design for drying chamber is shown in Figure 5-28. The sample bag made of material porous to gases but not liquids is inserted into the chamber. The bag is supported in a perforated metal basket to which heat is transferred from the outer shell via the support ribs. A simple twist-lock cap seals the container and a manual valve vents the container to space vacuum. After the allotted drying time, the spatial vent valve is closed and the cabin vent valve is opened to repressurize the chamber. The dried sample and bag are removed and stored in the emptied food storage area. Each chamber and accessory equipment will weigh approximately two pounds plus 0.1 pound per gram of sample. The amount of cabin atmosphere lost during each sample venting is negligible (0.0004 pounds). Therefore, the total system weight W is calculated from:

$$W = \underbrace{0.000618\ MS\ (2 + 0.1\ M)}_{\text{Chamber}} + \underbrace{MS\ \frac{(0.4\ gm)}{(454\ gm/lb)}}_{\text{Bag}}$$

$$W = MS\ (0.002116 + 0.0000618\ M)$$

For 1000-10 gram samples the system weight is:

$$W = 10000\ (0.002734) = 27.34 \text{ pounds}$$

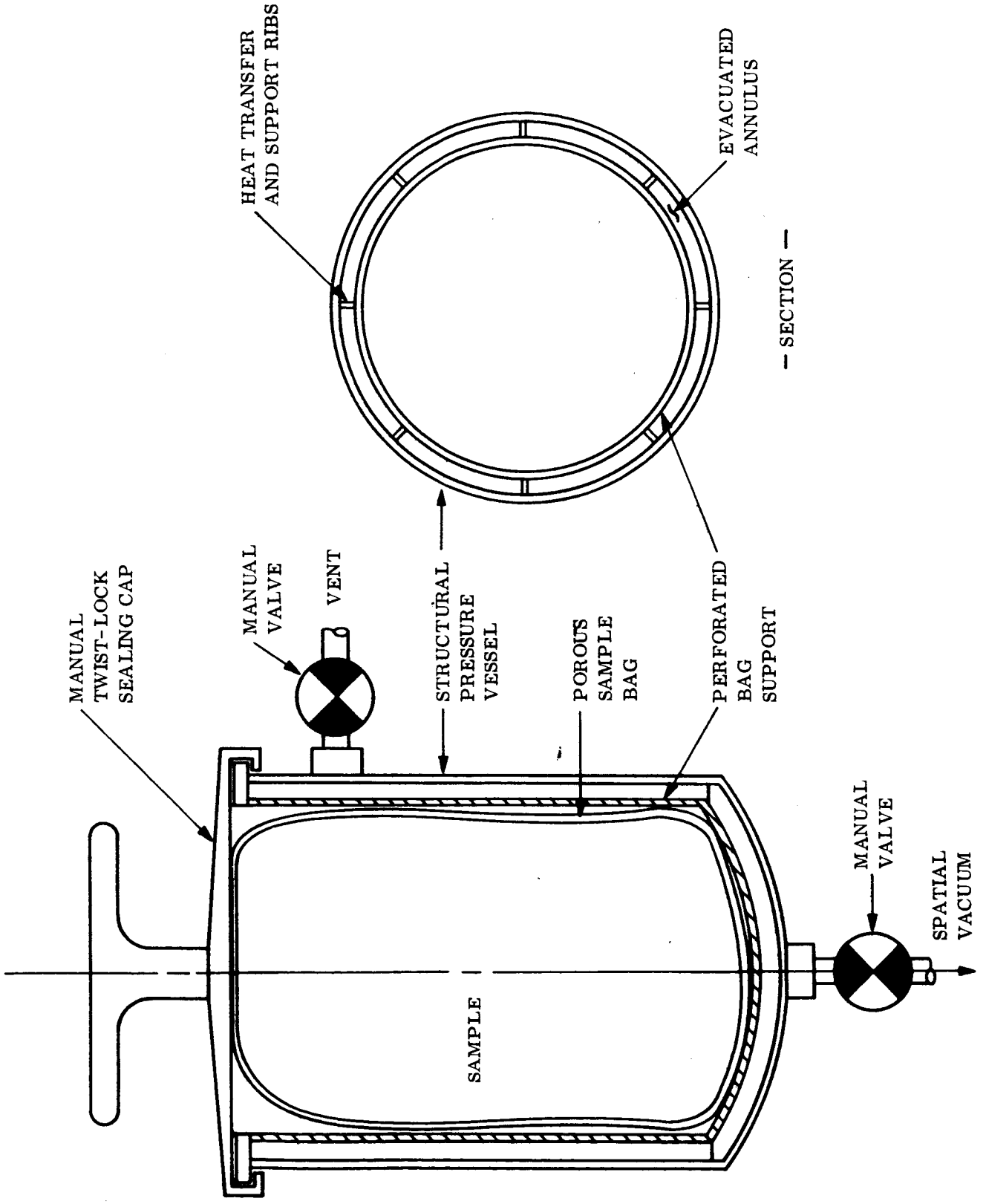


Figure 5-28. Conceptual Design - Drying Chamber

See Figure 5-29 for other weights.

The initial volume of the system is 0.1 cubic foot per chamber regardless of sample size between 5-20 grams and the bag volume is 4 cc per gram of sample including the packaging efficiency.

$$\underline{\text{Chamber}} \qquad \underline{\text{Bag}}$$

$$V = 0.000618 \text{ MS } 90.1) + \frac{4 \text{ MS}}{28,300 \text{ cc/ft}^3}$$

$$V = \text{MS } (0.0000618 + 0.000141)$$

$$V = 0.0002028 \text{ MS}$$

For 1000-10 gram samples the initial system volume is 2.028 cubic feet. See Figure 5-30 for other volumes.

5.3.5 ADSORPTION AND ION EXCHANGE

This technique of preservation is not recommended. See Paragraph 5.25.

5.3.6 LYOPHILIZATION

The approach to lyophilization which is recommended by this study is to utilize space vacuum for the whole process of freezing and subliming water vapor out of the sample. As in vacuum distillation, the sample will be connected to reduced pressure in two stages. Contained in some porous non-conductive material such as porous Teflon, the material will boil and cool until freezing occurs by means of the loss of heat in boiling (distillation). After freezing occurs, the pressure is lowered still further to sublime the rest of the water from a given sample. After sublimation of 95 percent to 99 percent of the water is completed, the sample is sealed in vacuo and can then be stored at cabin ambient temperature.

Lyophilization, or freeze-drying, is characterized by a sample temperature versus time function as shown in Figure 5-31.

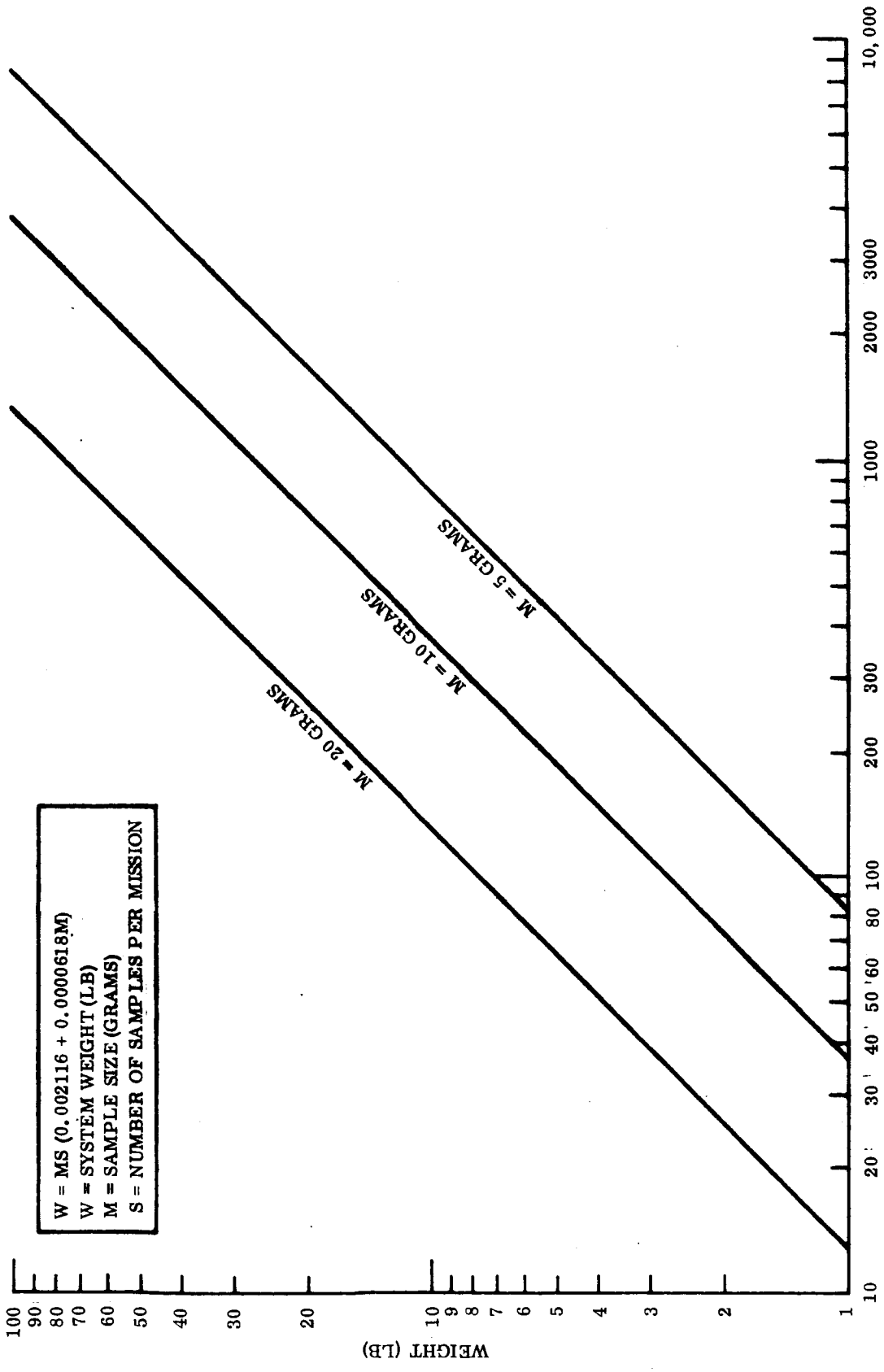


Figure 5-29. Preservation by Vacuum Distillation - Weight

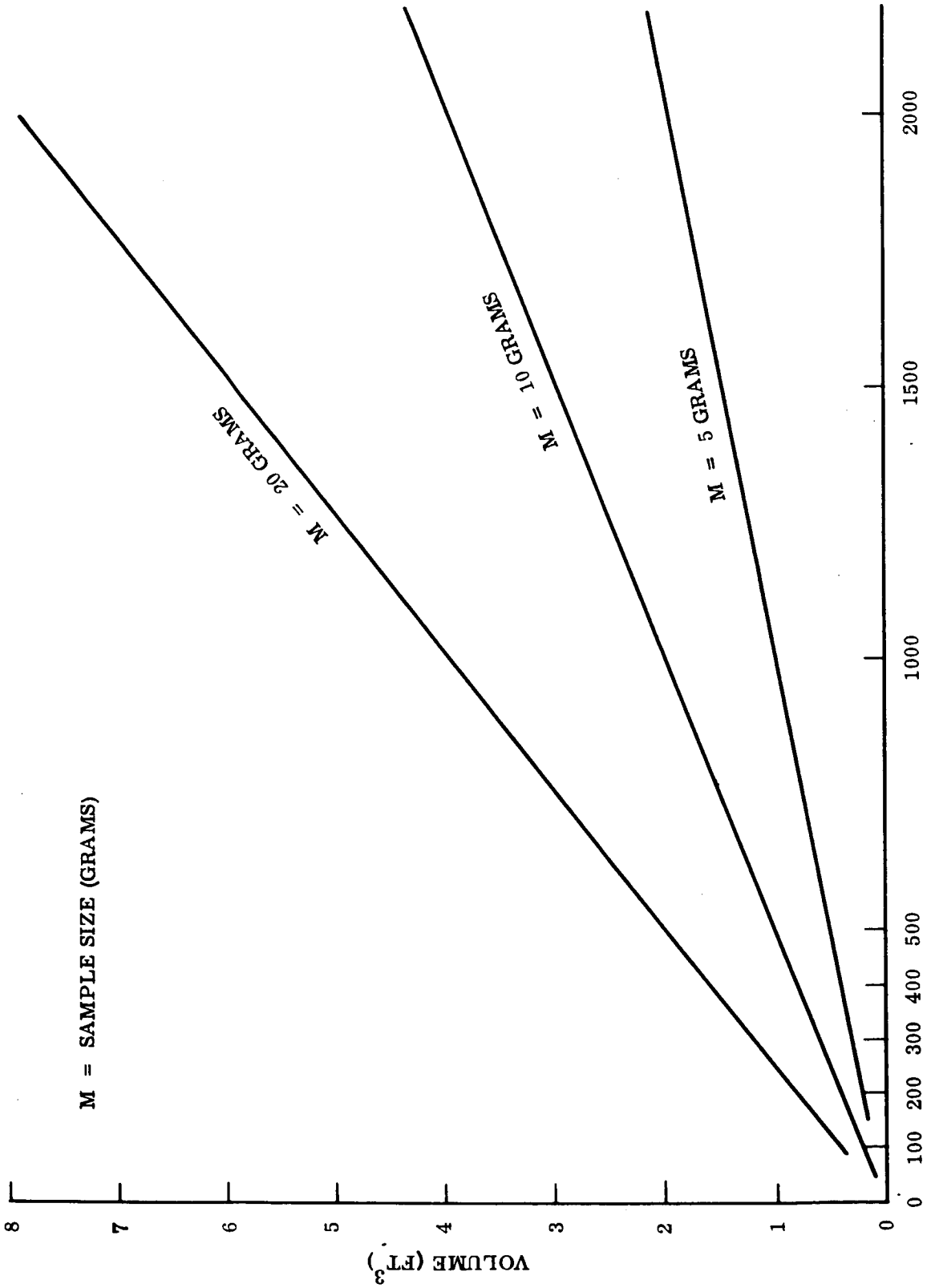


Figure 5-30. Preservation by Vacuum Distillation - Volume

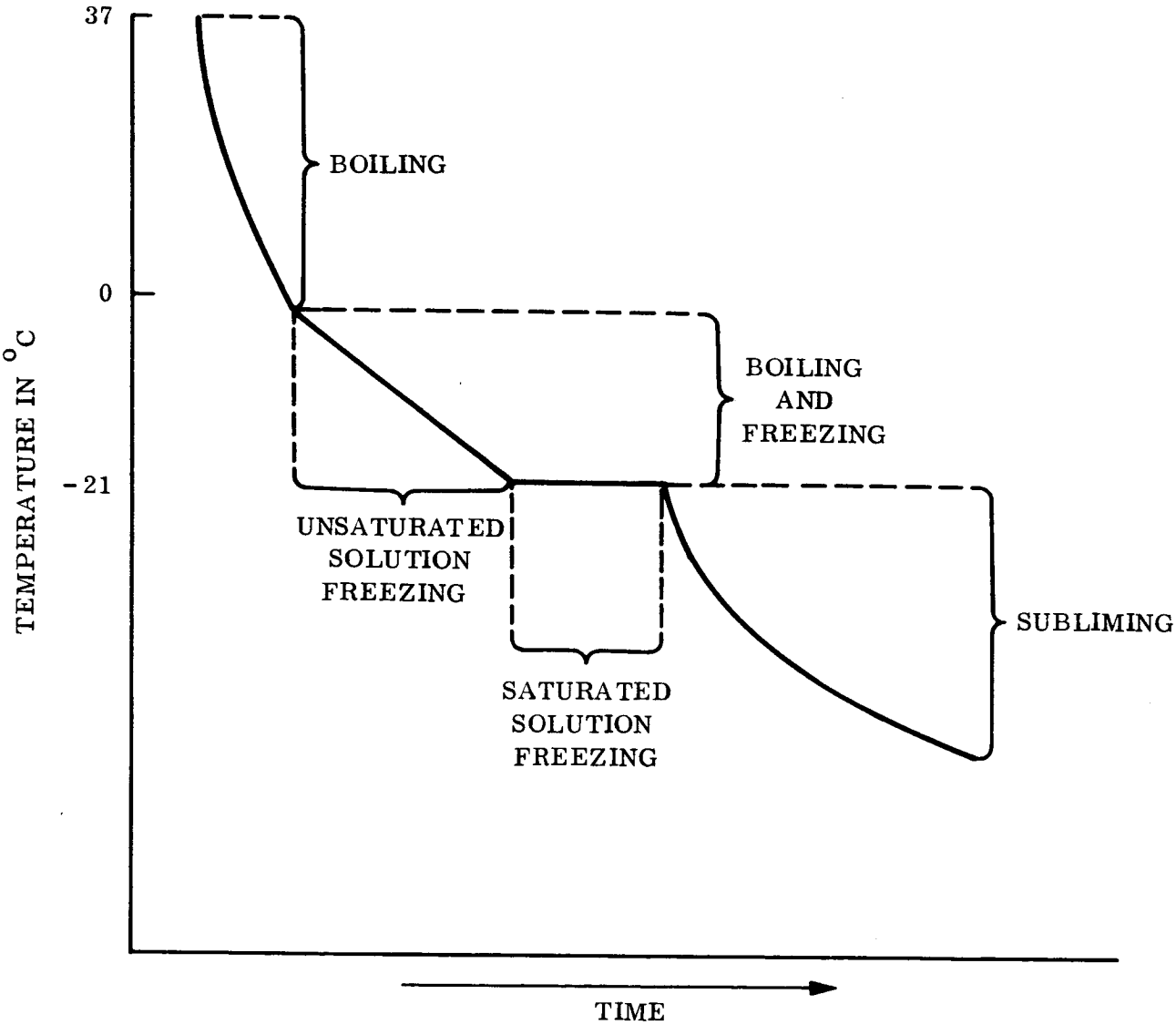


Figure 5-31. Thermal History for Vacuum Dehydration

The portion of the curve which represents boiling is very steep, because the high heat of vaporization of water causes the remaining liquid to be rapidly cooled. For pure water, the section of the curve corresponding to freezing would be a horizontal line. However, since we are dealing with an unsaturated solution, the line will slope downward as ice freezes out of the solution. This concentrates the solution, which in turn further depresses the freezing point. This gives a gradually sloping line until a saturated solution is reached, after which time ice and the salts in solution will freeze out together at one temperature as long as any liquid is left. After all of the specimen is frozen, the temperature will again decline rapidly as the subliming ice will withdraw both the heat of vaporization and the heat of fusion from the remaining sample. As the latter is now frozen, it can no longer furnish the heat of fusion which produces the horizontal portion of the thermal history curve.

Initial laboratory investigation of vacuum drying urine and feces slurries were unsuccessful because liquid was forced through a porous material used for liquid containment when the vacuum was applied, resulting in the loss of part of the specimen. The material used was porous Teflon, and water would not pass through it until a pressure several tenths of a pound was applied. The vapor pressure of water at $37^{o}C$ ($98.6^{o}F$) is 0.91 psi, or more than enough for the vapor formed to push the liquid through the porous Teflon. This condition was prevented by orificing the vacuum line to restrict the flow of vapor. The liquid specimen still boiled, but if the restriction in the vacuum line caused a much larger pressure drop than the flow of water vapor through the porous container, the pressure created by the latter flow would not be enough to force the liquid through porous Teflon.

The restriction in the vacuum line will no longer be required once the freezing portion of the curve is reached, because the vapor pressure of water in equilibrium with ice is 0.0886 psi, and this is not enough to force the liquid through the Teflon material. However, the slowest part of vacuum dehydration will be that represented by the sublimation portion of the curve, due to the fact that the sample can no longer provide the heat of fusion to vaporize more of itself. The only heat to vaporize the remainder of the water will come from conductive flow to the sample. If the sample is contained in a poorly conductive material, the heat flow to the

sample will be the limiting factor in completing the dehydration rather than the orifice in the vacuum line, Evaporation of 18 percent of the water will be sufficient to cool the remaining 82 percent from 37°C (98.6°F) and freeze it. Thus sublimation will have to remove 82 percent of the water from a specimen.

The recommended approach to preservation by lyophilization is to retain the sample in a porous bag and subject it to space vacuum. This preservation technique is similar to preservation by vacuum distillation. The only difference between the two techniques is the rate of heat transfer into the sample container which is varied by the mechanical design.

Based on test data (25), the time to dry a sample by lyophilization is approximately twice as long as that required for vacuum distillation of 20 minutes per gram of sample and 25 percent utilization of chambers. See Figure 5-32 for typical test data. Therefore:

Chamber	Bag	
$W = 0.001236MS\,(2 + 0.1\,M) + MS\,\frac{(0.4\ gm)}{454\ gm/lb}$		Where:
		W = System Weight (lb)
		M = Sample Size (gm)
$W = MS\,(0.003352 + 0.000123M)$		S = No. of Samples
		V = Volume (ft^3)

For 100-10 gram samples the system weight is:

$$W = 10000\,(0.004582) = 45.82 \text{ pounds}$$

See Figure 5-33 for other weights.

$$V = 0.001236MS\,(0.1) + \frac{4MS}{28,300}$$

$$V = 0.0002646MS$$

For 1000-10 gram samples the initial system volume is 2.646 cubic feet. See Figure 5-34 for other volumes. No electrical power is required since all controls are manually operated.

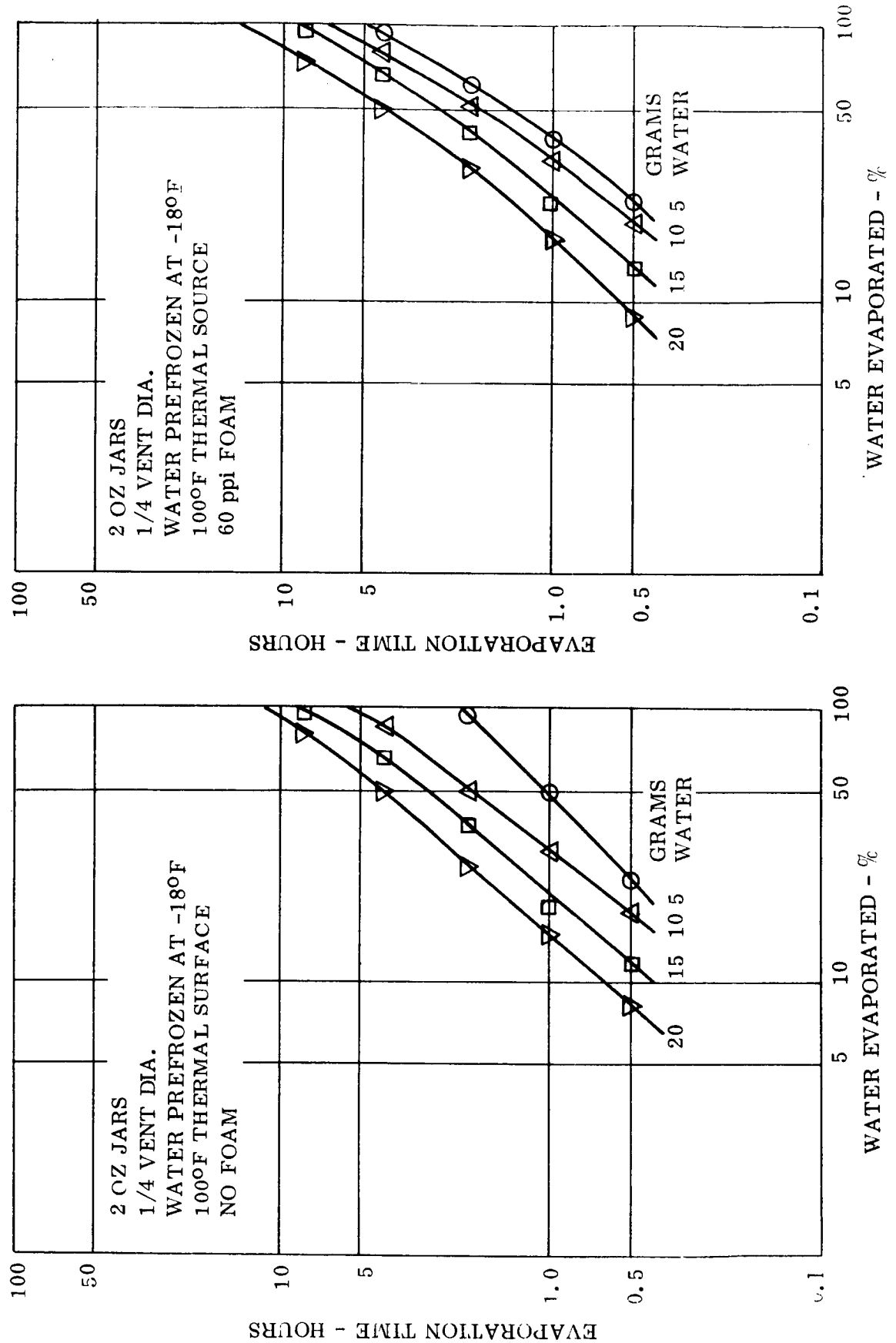


Figure 5-32. Typical Lyophilization Test Data for Prefrozen Samples

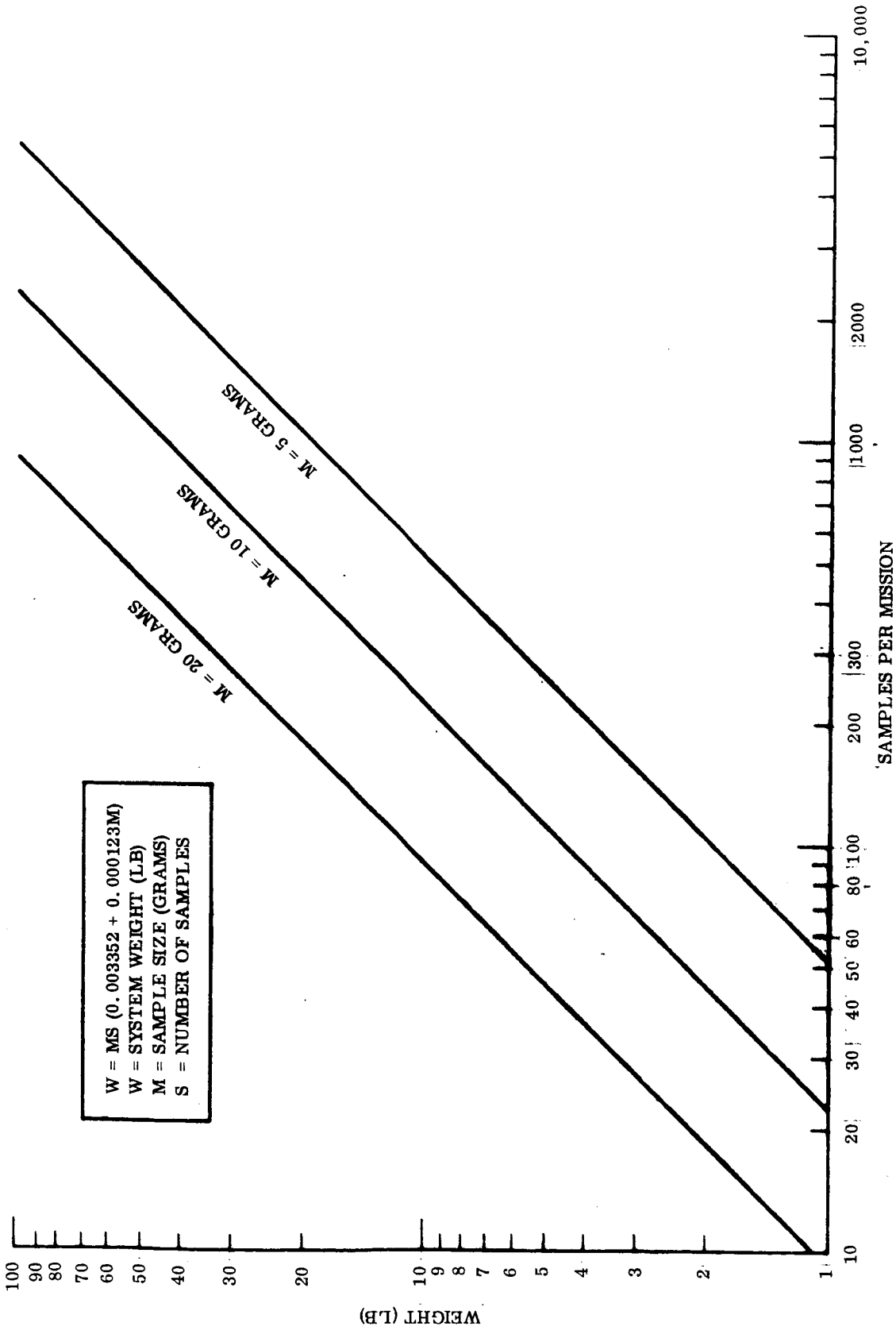


Figure 5-33. Preservation by Lyophilization - Weight

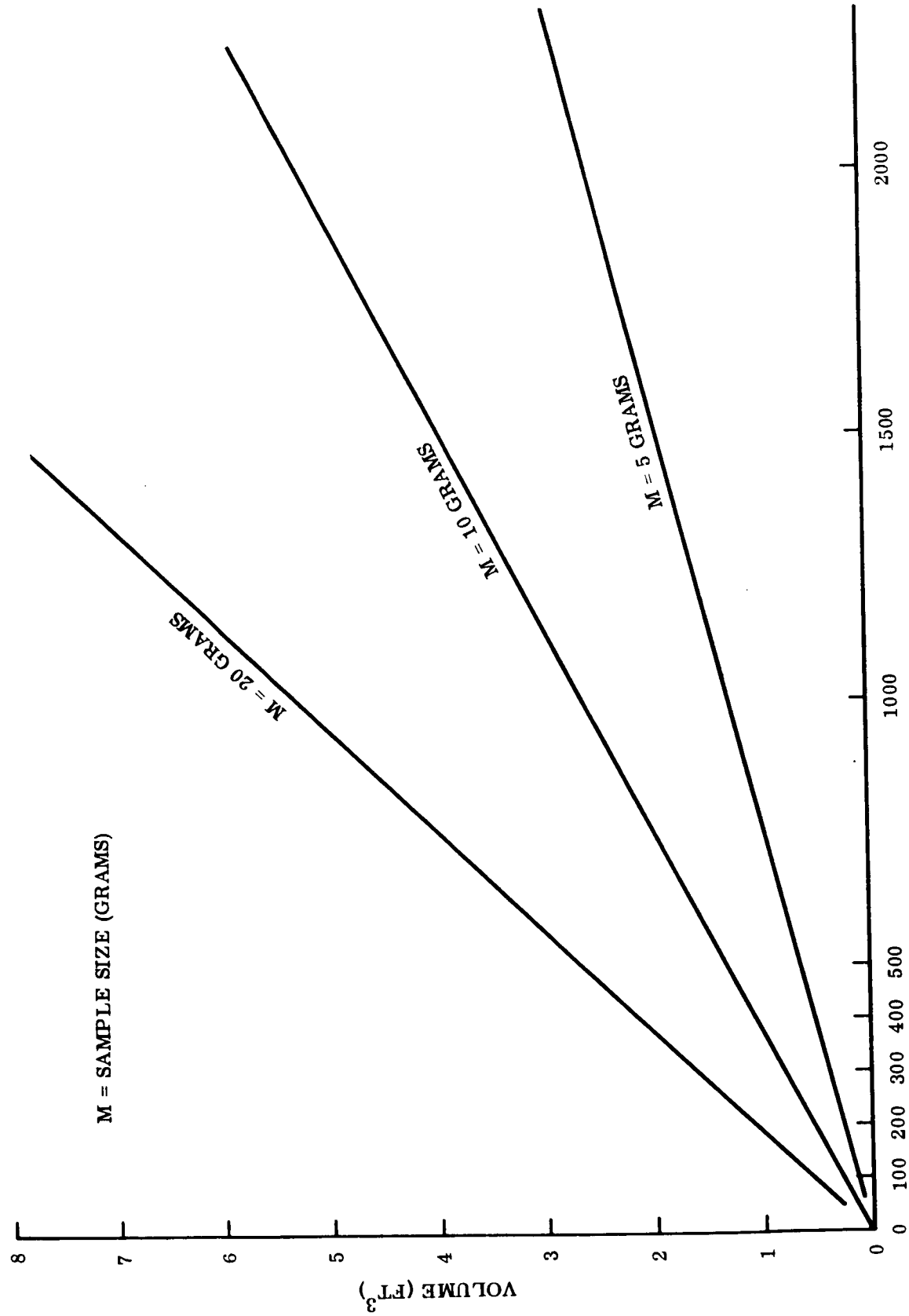


Figure 5-34. Preservation by Lyophilization - Volume

5.4 BIOLOGICAL ASPECTS OF PRESERVATION

Although there are a few studies in the literature concerning analyses on stored samples of whole blood, serum, plasma, urine, feces, and sweat, and most reference books on clinical biochemistry do briefly discuss sample preservation, only a few studies have considered long term storage under various well defined storage conditions as an experimental variable. Because of the paucity of data, recommendations for the use of one or another of the preservation techniques described are based largely on what, we hope, is a logical use of available data, past experience, and considered opinion.

Preservation of samples could be accomplished for the vast majority of components by the use of appropriate chemicals. However, because of problems of incompatibility, interference, handling, etc. a different chemical might be most ideal for each component in each biological material. Preservation by refrigeration, freezing or lyophilization, which would be of general applicability, might therefore be preferable to several specific techniques. The extra launch weight, coding and storage complexities and potential hazards involved in the use of chemical preservatives, which are not usually innocuous materials, might also mitigate against the choice of this method.

The range of storage temperatures for the various samples is shown in Figure 5-35.

There are major advantages in using a single preservation technique for all samples of all biological materials. First of all, the ease of performance would be greatly increased if astronauts only had one method of preservation to use. They would require greatly simplified training, and the activities with respect to storing samples would be limited to a single set of operations. Second, the different kinds of equipment which would have to be installed in a spacecraft would be limited to a single species, greatly reducing volume and weight penalties due to elimination of a series of preservation techniques. For example, if freezing at one temperature were the method of choice, it is possible that a single freezing compartment could be built into the spacecraft with one set of walls rather than a multiplicity of compartments: one for refrigeration, several for

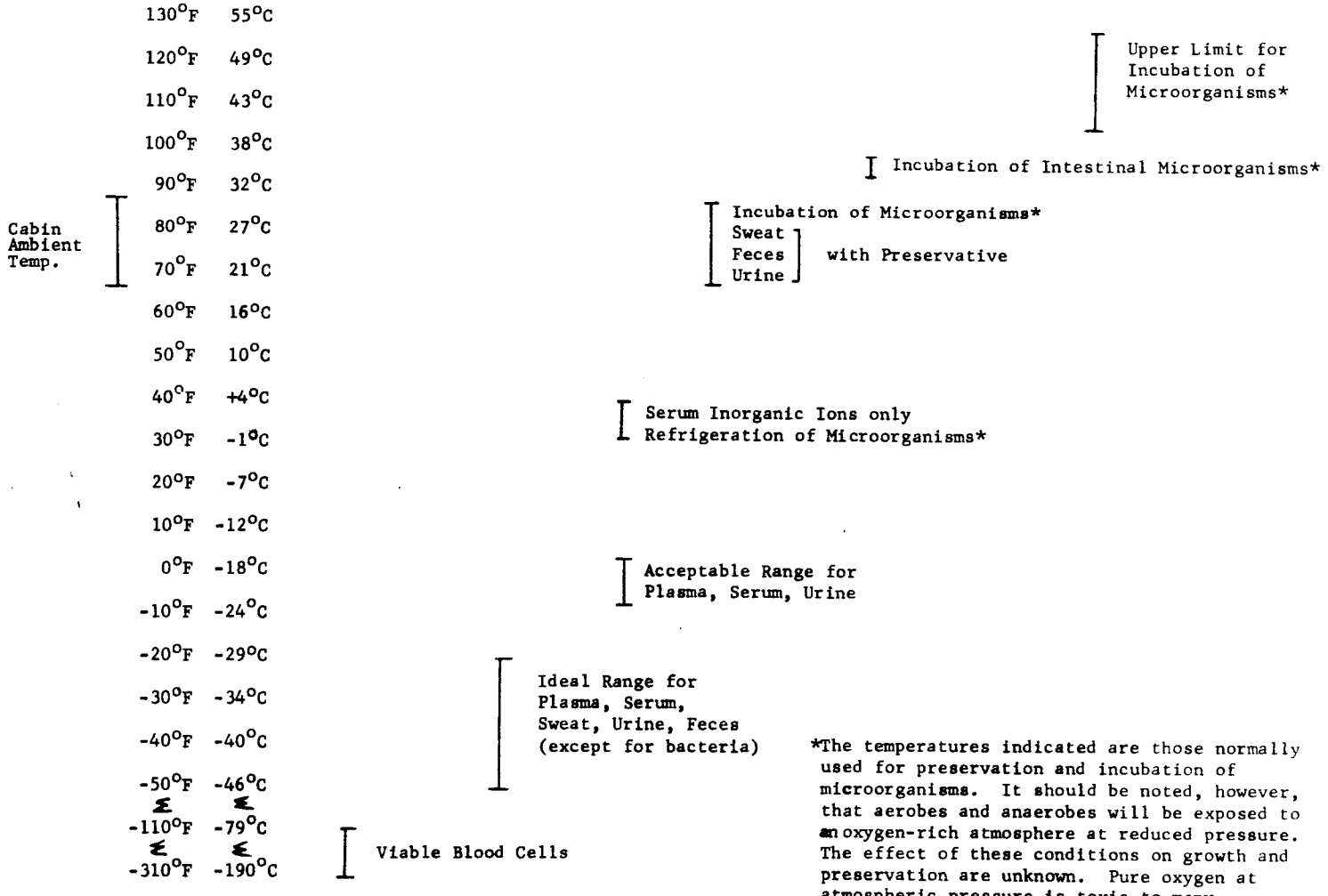


Figure 5-35. Temperature for Sample Preservation

freezing at different temperatures, one for storage at cabin ambient temperature and several for incubation at higher temperatures. Third, modules for transfer of samples during resupply would be limited to a single type and possibly even to a single container if it could be made large enough to handle all samples collected between dates of resupply.

However, the idea of using a single preservation technique is wishful thinking. It becomes obvious when considering the various biological materials, i.e., blood, urine, sweat, feces, and microorganisms, and especially when considering the stability of constituents of the materials using any one preservation method, that a single preservation method has distinct disadvantages which overshadow the advantages. No single preservation method will preserve all the constituents of interest. Freezing or lyophilization could be used for preserving all urine constituents which are distinct chemical entities. Freezing or lyophilization could also be used for feces and sweat. Lyophilization, however, would require preliminary measurement of the volume or weight of samples on the spacecraft so that concentrations of constituents existing at the time of withdrawal could be reconstituted by the addition of water when samples are assayed in an earth-based laboratory. (Lyophilization would also lead to loss of volatiles other than water, as mentioned elsewhere). Such measurement of volume and/or weight presents major difficulties in a zero gravity environment. But when it comes to blood and microorganisms, it becomes obvious that a single preservation method would have distinct disadvantages. Freezing could not be used for microorganisms since it destroys many of them. Refrigeration and probably incubation would be required for samples of microorganisms. With reference to blood, separation of plasma or serum from cells would still be required for analyses of certain components regardless of the type of preservation chosen. Then too, unless freezing to low temperatures ($< -79^{o}C$) with protective adjuvants and controlled rates were used with consequent major engineering difficulties, no one technique of preservation could be suitable for all blood constituents. Some constituents would still require chemical preservation and/or on-board determination if freezing at higher temperatures were chosen. Lyophilization cannot preserve the formed elements of blood. Chemical preservation

cannot be used for all materials because of lack of stability of some constituents.

In summary, a single technique of preservation would be advantageous from several points of view. Such a choice cannot be made, however, because stability of all constituents of biological materials cannot be insured by any one preservation method.

5.4.1 BLOOD PRESERVATION

The preservation of blood for later clinical analysis is a problem which has received little study because in routine practice blood tests are performed within a very short time after withdrawal of a sample. As a result, there is a dearth of information on the subject in the literature. The information which exists is for the most part derived from studies on the banking of blood and plasma for later transfusion. This is a different problem from the one at hand; and, although some information falls out of such studies, they usually concern themselves with the effect of stored blood on patients rather than recovery of specific blood components for clinical analysis. The major exception to this is the study of recovery of <u>viable</u> blood cells; this, however, is not the same as recovery of <u>countable</u> blood cells. Because of this lack of information conclusions concerning the feasibility of various techniques of preservation must be viewed as tentative. Definitive conclusions can only come from an extensive laboratory study of the problem.

In most cases the best method of storing serum or plasma is freezing. This requires immediate separation of cells from fluid after withdrawal from subject by allowing clot to form (serum), rimming of clot (serum), centrifugation of sample (serum and plasma), and closing of clips or valves between cells and fluid in a compartmentalized container used for withdrawal and storage. Rimming of the clot may present difficulties in a zero gravity environment where the behavior of liquids does not allow the opening of containers and introduction of an applicator stick to loosen the clot from container walls. Some kind of rimming device might have to be built into the container (Figure 6-12) or an alternative could be sought in a laboratory study.

In cases where samples are frozen, microbial contamination should not produce problems. To avoid microbial growth in those few studies where freezing is not otherwise required and antimicrobial chemical preservatives are not used, it may still be desirable to freeze samples.

Where anticoagulants or other chemicals must be added to the samples before storage and are not already present in collection containers, they must be added using techniques which do not require the opening and closing of containers of liquids. A method of avoiding this problem is to employ syringes and needles, and containers stoppered with rubber septa for both blood collection and reagent storage. The reagent bottles could then be entered with the needles without spillage of contents due to zero gravity. Similarly, reagents can be added to blood in containers by penetration of septa with a needle and injection of contents of the syringe.

Determination of volume of sample where this is required prior to storage can be done by withdrawing blood into calibrated syringes (e. g., blood lactic acid).

Since more than one method of blood collection,handling and preservation must be employed, a sampling protocol will have to be developed to obtain aliquots in the different ways necessary on a rotating basis from day to day. This protocol is apt to become quite complex because of the variety of collection and handling techniques as well as preservation methods necessary. Therefore, a relatively simple sampling protocol is strongly recommended, at least for the first series of missions.

5.4.1.1 Suitability of Preservation Methods

a. Time for Which Sample Can Be Stored Without Jeopardizing the Reliability of the Subsequent Analysis (within standard laboratory accuracy)

In most clinical laboratories, analysis of blood is carried out within a few hours of collection. For most assays, studies have not been performed to determine the feasibility of measuring blood components after prolonged preservation with the result that no standards are available for such measures.

A preliminary laboratory investigation would be necessary to determine standard normal values of measurements on blood stored in various ways over a period of time equal to that during which samples would be stored in flight.

b. Effect of Weightlessness on Collection and Preservation Technique

New techniques may have to be developed for collection and preservation. Since foaming may result when fluids are collected by vacuum aspiration, the techniques for collection of blood used on earth must be tested in flight. A method employing a collapsed sack within a rigid container which could later be centrifuged and partitioned, and then refrigerated or frozen might have to be devised. This sack would be filled by pressure in a vein following venipuncture. It would have a valve positioned so that all cells would be in the bottom section following centrifugation. The valve would then be closed, and the whole container could be frozen or refrigerated without fear of contaminating the supernatant serum or plasma with cells or their breakdown products.

For blood smears, and on-board clotting time and microhematocrit determination, capillary action would be adequate to fill the capillary tubes necessary and to transfer drops of blood to glass slides following puncture of a finger, toe, or ear lobe.

c. Complexity of Sample Preparation for Preservation

No one sample technique would be suitable for all blood samples. Centrifugation would be necessary in most cases. The complex freezing techniques for preserving formed elements would probably prevent these methods from being useful.

There are five main methods available, one of which is overly complex: (1) blood smear from finger puncture; (2) filling and centrifugation of capillary for microhematocrit from capillary puncture; (3) chemical dilution and preservation for cell counting; (4) filling of collapsed sack and compartmented chamber by venipuncture, followed by centrifugation and closing of chamber locks, followed by uncontrolled freezing in a unit maintained at -20°C (-4°F); (5) withdrawal of blood by venipuncture into a syringe and transfer from syringe to a tube followed by subsequent processing (e.g., centrifugation, removal of serum or plasma, etc.)

d. Special Biological Problems

1. Use of Anticoagulants - Anticoagulants are used routinely in collecting blood for analysis. The only danger would be that during collection small quantities might be introduced into the blood stream of astronauts. This problem can be avoided by using syringes for withdrawal which do not contain anticoagulant followed by immediate transfer to tubes or containers which do or by using a compartmented container containing a collapsed sack in one section and anticoagulant in a second section closed to the astronaut's blood stream, but which could be opened and mixed after withdrawal of sample is completed.

2. Hemolysis - Hemolysis cannot be avoided completely by any technique of preservation of whole blood. Because of this problem and the resulting contamination of serum or plasma which can vitiate other analyses, it would be necessary to separate erythrocytes from plasma or serum by centrifugation and either transfer plasma or serum to another container or close a valve separating cells from supernatant in a compartmented container.

3. Protein Precipitation - In certain cases, preservation of serum, plasma, or whole blood after precipitation and removal of protein may be necessary (e. g. blood lactic acid). In other cases, precipitation of protein may not interfere with subsequent tests (e. g., sodium, potassium and other cations). In still others, the technique contemplated for blood preservation should not precipitate protein.

4. Contaminant Microbial Growth - Since blood will be collected aseptically in sterile containers, heavy microbial contamination should be avoidable. Refrigeration, freezing and chemical preservation will slow or prevent microbial growth.

e. Complications Due to Addition of Common Ions in Chemical Preservation

Tests for constituents of blood must be performed on samples containing no added interfering ions in the form of ions being determined or enzyme inhibitors in anticoagulants or other sources. Separate samples for some blood tests might thus be necessary. For example, plasma preserved with sodium oxalate should not be used to determine sodium concentration of blood.

5.4.1.2 Feasibility of Preservation Techniques

a. Chemical

Chemical methods for preserving blood for later analysis are usually not effective for long-term storage (26). Certain tests can, however, employ chemically preserved blood: 1) red blood cells may be able to be preserved with little loss, in formalin, for later cell counting (27); 2) it may be possible to store white blood cells for counting using a modification of this technique (27); 3) blood smears made during flight may be preserved by drying with subsequent fixation.

Formaldehyde was one of the first preservatives used for blood. It fell into disfavor, however, when it was discovered that commercially available formalin reacted with alkaline copper tartrate in tests for glucose. Higher grades of formalin now available may not do this, but the problem has not been re-investigated. Formalin, however, interferes with some analyses such as urease measurement of urea (26). Fluorides have also been used and, in sterile blood can preserve glucose, urea, non-protein nitrogen, creatinine, cholesterol and

uric acid in a stable state for as long as ten days at room temperature. It is thought that fluorides exert their preservative effect by inhibiting certain enzymes such as those involved in glycolysis. For the same reason, many analyses which employ enzymatic reactions are vitiated by the use of fluoride. By combining thymol as an antibacterial agent with fluorides, non-sterile specimens can be preserved for all of the above constituents except non-protein nitrogen for at least two weeks. Monochlorobenzene and monobromobenzene have also been used in conjunction with fluorides instead of thymol. High concentrations of fluorides are apt to produce hemolysis, however (26). None of these agents could be used to preserve constituents for bioassay due to toxicity.

These preservatives have limited usefulness for the purpose of long-term preservation, however, and they interfere with some constituent analyses. In addition, they are extremely toxic to man and would present hazards to a space crew. The use of formalin for red and white cell preservation as mentioned in the preceeding paragraph would have to be carefully controlled to assure crew safety. This is true, also, for fixatives which might be used to preserve blood smears.

Antibiotics have also been used as antibacterial agents and have been used to preserve blood for hemoglobin and urea measurement, but duration of stable storage is not known. The stability of other constituents using this approach is also open to question (26).

Because of the potential hazards presented by chemical preservatives, great care must be taken in packaging these chemicals and transfering them from one container to another. This is particularly difficult on a spacecraft where the force of gravity cannot be depended upon to keep materials in storage containers when they are opened for transfer. It would be very difficult to develop "fail-safe" techniques for handling preservatives; therefore, a thoroughly trained crew is essential. A possible approach would be the use of syringes and needles for transferring fluids through rubber septa in storage and sample containers. Wherever it can be applied, the use of pre-treated containers is recommended. In this case, the only problem which remains is that of introducing the sample into such a container without loss of preservative. The use of sealed compartments containing the preservatives might be the answer. The sample would be introduced into an outer compartment and the container sealed to the outside. Then an inner sealed compartment could be opened by manipulation of a valve or use of pressure. The sample and preservative could then be mixed safely. Details of some techniques for specific constituents are given elsewhere.

b. Refrigeration (Chilling)

This method is useful only for short-term preservation of whole blood (1-3 weeks). Some blood components of interest will not be preserved, e.g., phosphates, sulfates. The technique requires the addition of anticoagulants and possibly other

chemical preservatives (28). Many components of plasma or serum would be quite stable if refrigerated, provided that microbiological contamination was prevented (29).

c. Freezing (various temperatures)

Rapid freezing and storage of whole blood at -79° C (-110.2°F) or less is a potential method of preservation for some components of interest (30 - 49). One technique calls for the addition of sugars and/or polyvinylpyrolidone with quick freezing (uncontrolled rate) and storage at liquid nitrogen temperatures (-190° C) (35, 36). Another technique uses glycerol or dimethylsulfoxide as a protective agent (30, 37). Cooling rates must be controlled, however, and the usual temperature of storage is -79° C (-110.2°F) or less. Modification of this technique might permit storage at temperatures as high as -20°C (-4°F). No technique permits complete recovery of formal elements.

Plasma and/or serum separated during flight may be frozen at uncontrolled rates and stored at -20°C (-4°F) with little if any change in most components of interest for periods of many months (50).

d. Vacuum Distillation (Drying)

This method is not applicable to the preservation of whole blood; many components of plasma or serum could be preserved indefinitely in the dry state if the mechanical problems of vacuum distillation were overcome (29).

e. Adsorption and Ion Exchange Technique

This technique is not applicable to the preservation of whole blood. It might be useful for a few studies on inorganic constituents of serum, but much simpler methods of storage and preservation are available for the same parameters. No citations concerning the use of this technique have, to date, been discovered in the literature.

f. Lyophilization

Lyophilization is not applicable to the preservation of the formed elements of blood. Fractions of cells recovered using this technique are too small to be used (40). From this point of view, lyophilization is inferior to the low temperature freezing techniques described elsewhere. It is not feasible to use these low temperature techniques for blood because of the engineering penalties imposed and the variable recovery of formed elements obtained. Other alternatives for preserving formed elements are suggested.

From the point of view of preserving chemical and biochemical constituents of plasma and serum, lyophilization is an excellent technique. Many components are preserved almost completely unaltered for a period of years (28). Refrigeration at 4°C is helpful in increasing the period of storage without constituent changes.

Lyophilization, however, entails the most severe weight and volume penalties of any technique. Freezing of plasma and/or serum as in (3) above, is just as effective for flights of several months duration, and, because of the reduced engineering penalties using this method, freezing is recommended above lyophilization as the method of choice.

Another drawback to lyophilization is the length of time required for performance of the initial drying process. This technique would occupy an astronaut for periods of time lasting many hours in order to ascertain when an adequate level of dryness is obtained. Freezing, on the other hand, is a much quicker process and will not require monitoring by an astronaut. Aliquots of biological materials will simply be transferred to a freezer for uncontrolled freezing or to a cold finger device if more rapid freezing is desired.

g. Combination of Chemical and Other Methods

All refrigeration or freezing techniques for whole blood requires the prior addition of chemicals as preservatives and/or protective agents. Some refrigeration but few freezing techniques for serum require the same.

Some of the variables listed in Appendix A cannot be measured on blood stored in any manner, e. g., clotting time, clot retraction, platelet adhesiveness. Others can only be measured readily on aliquots stored using chemical preservatives.

The use of chemical preservation in combination with other methods for plasma and serum is limited for the most part to a combination of chemical preservatives plus refrigeration. Combining chemicals plus freezing, vacuum distillation or lyophilization is not discussed in the literature as far as we can ascertain. These latter techniques when applicable seem adequate by themselves. Duration of stable storage in the frozen state might be increased by the use of chemicals, but this is an area where further study would be required since no information was found concerning this approach.

The use of chemical preservatives plus refrigeration is known to prolong the period of stability of many blood constituents. The total period of storage possible is still limited, however, to two or three weeks. Stability is thus prolonged from days for either method to weeks. This period is not long enough for periods of resupply contemplated which would be 45 days or longer. A laboratory study would be required to establish the feasibility of using chemical plus refrigeration storage for blood, serum or plasma. Such a study could also attempt to develop new variations of this technique with prolonged storage if possible. As has been reiterated several times, there is a dearth of information in the literature on the problem of stability of biochemical constituents of blood stored in any way.

h. Other Methods

Drying plasma or serum under vacuum in the presence of a desiccant is a possibility requiring considerable research and development. It presents many difficulties, but on the other hand offers the attractive prospect of indefinite stability for many plasma and serum components with storage at cabin temperature.

5.4.2 URINE PRESERVATION

On-board urinalyses are not feasible at the present time due to payload restrictions. Many of the urine constituents are present only in minute amounts and require sophisticated and complex procedures for their detection. For these reasons, emphasis has been placed on urine preservation during space flights and post-flight analysis by trained specialists in earth laboratories.

The problems of urine collection and preservation have been described as "prosaic" (51). This can be defined as dull, commonplace, or humdrum. A literature search does little more than emphasize the paucity of information available and points up the need for a comprehensive laboratory research program on urine preservation. For the most part, the present state of the art of urine preservation is a product of earth-bound clinical laboratories whose restrictions, needs, and facilities are quite different from those in a spacecraft on a prolonged space flight.

A literature survey has shown that two methods have been available to preserve urine constituents when immediate analyses are not possible. They are: treatment of the urine with chemical reagents and lowering and maintaining, the urine temperature near or below its freezing point (26), (28), (52), (53), (54). "Chemical preservatives are used on the supposition that bacteria in urine are primarily responsible for the decomposition of its constituents and preservation can be accomplished by limiting the concentration of these microorganisms in urine" (51). Reduction in temperature aids in preservation by reducing bacterial growth rate. Reaction rates of oxidation, glycolysis, hydrolysis, and other decomposition reactions are reduced by temperature reduction. In general, non-enzymatic reaction rates vary by a factor of 2 within 10^{o}C temperature intervals. If urine is main-

tained in the solid state, further reduction in reaction rates occur because of diminished diffusion rates and specie interaction.

The fact that urine contains such a large number of diversified substances contributes to the problems of adequate preservation. Techniques have evolved as by-products of clinical research programs where a definite need for preservation of only a few urine constituents was indicated. As a result, the preservation methods are highly specific with regards to constituents and their effectiveness has only been evaluated for relatively short time periods.

Preservation Methods

Chemical

A large number of chemical reagents have been recommended as bacteriostatics or bactericidals (26), (28), (52), (55), (56). These often serve for interference-free detection of a single constituent. A universal preservative has long been sought, but no such material is yet available. Several suggested compositions, although suitable for relatively short storage periods and routine analyses, do not apply in the case of special assays or for certain specimens containing unusual quantities of pathologic constituents (28). Literature surveys reveal little information concerning chemically preserved urines of long storage duration (30 to 60 days).

Some classical agents promote the stability of several biochemical constituents and at the same time accelerate the decomposition or interfere with the available bioanalytical methodology for others (55). Boric acid is normally considered to be an acceptable stabilizer for many clinically important constituents, but it interferes with catecholamine analyses. Hydrochloric acid addition to pH 2 is a good preservation method for 17-hydroxycorticosteroids (55) and serotonin (52), but this low pH can denature albumin and other proteins and thus interfere with certain analyses for these important constituents. Acidic conditions can also accelerate hydrolysis of labile organic constituents and precipitate uric acid salts. Other salts have been observed to precipitate under acidic conditions resulting in absorption

of calcium, phosphate, and protein. It is speculated that other organics may be adsorbed also (55). Alkaline pH conditions arising from preservation methods or bacterial action favor calcium phosphate precipitation. Here again, there are possibilities of constituent adsorption, and unless these phenomena are carefully considered during subsequent analyses, gross errors in determinations will result. Glucose, catecholamines, and 17-hydroxycorticosteroids are generally considered the most labile constituents under alkaline storage conditions (55). The decomposition is believed to occur mainly through redox mechanisms. Control of pH and microbial growth, plus the action of antioxidants inhibit degradation of these compounds.

Toluene is commonly used as a preservative in clinical practice but it is not effective against bacteria or mold already in the sample (28). A thin layer over the urine surface prevents contamination from external sources. This could be accomplished just as readily by closed containers. Toluene is a nuisance in pipetting and can extract small quantities of organics from the urine. Furthermore, gravity is required for this preservation method.

Chloroform, phenol, thymol, formalin, and benzoic acid have been used as preservatives for short storage periods (28), (52). Antimicrobial activity is varied and the degree of interference with special tests has not been ascertained. Chloroform interferes in the test for glucose as does formalin which also interferes with the Obermayer test for indican. Formalin is said to form a precipitate with urea which acts as a nuisance in microscopic examinations. Thymol is believed to interfere with tests for albumin and bile while both thymol and phenol may interact in colorimetric procedures involving oxidative steps (52). A combination of acetic acid and formaldehyde can be used for aldosterone. Benzoic acid was used to preserve urine during the Gemini VII flight of fourteen days duration for postflight analysis of 17-hydroxycorticosteroids, sodium, potassium, chloride, calcium, phosphate, magnesium, sulfate, nitrogen, creatinine, aldosterone, and catecholamines. However, the existing reports (68, 69) do not discuss the efficacy of this type of preservation. A recent laboratory study of urine preserved with hydrochloric acid for 30 days is noteworthy (56). Four urine constituents were selected to determine effectiveness of the method.

They were calcium, urea, 17-hydroxycorticosteroids, and catecholamines. Human urine samples were acidified to pH 2 with hydrochloric acid and stored at 24°C (75.2°F). Considerable hydrolysis was noted as evidenced by sample coloration. After 30 days, no viable bacteria were detected by means of microscopic or pour plate examinations. There were no changes in content of the four constituents tested.

In parallel experiments, urine samples were acidified to pH 3. After 30 days, the pH was found to be 8.0. Untreated urine samples (initial pH 5.6) had pH values around 7.5 after 30 days. Profuse bacterial and fungal growth appeared in both urines after four days. Hydrolysis was not observed in either the treated or untreated samples. Unfortunately, data as to constituent degradation were not given.

New chemical agents have been suggested and some have been tested as urine preservatives (55). As with the older preservatives, specificity of constituent protection and interference with analytical procedures are problems that still remain to be solved.

Antioxidants such as butylated hydroxy-toluene or butylated hydroxy-anisol have been suggested to stabilize constituents sensitive to oxidation (55). These compounds have also proven effective as antimicrobial agents and thus could possibly act in a dual role in urine preservation. Other potentially good preservatives include ethyl and propyl gallate or alkyl-p-hydroxybenzoate.

A recent laboratory study has isolated a mixture of inorganic materials which were found to be very effective in preserving catecholamines, 17-hydroxycorticosteroids, glucose, and creatinine in urine during a 14-day storage period at ambient temperatures (55). No data were provided as to interference with other analytical procedures. The mixture consisted of sodium fluoride, sodium metabisulfite, and sodium borate. Excellent preserving action is attributed to the anti-microbial activity of fluoride, anti-oxidant properties of metabisulfite, and the maintenance of acid pH conditions by the borate.

All of the preservatives mentioned are toxic to man to a greater or lesser degree. The problems of handling these in space flight are similar to those for the chemical preservation of blood, and the same comments apply. Whether or not one chemical preservative is more toxic than another is a moot point with reference to this application because any degree of toxicity presents a hazard. If any of these chemicals are spilled during space flight, there is no easy mopping up procedure in zero gravity short of venting the cabin. They are all irritants to the skin and eyes, and poisonous if inhaled or ingested in varying amounts.

The state-of-the-art of preserving urine chemically for extended periods of time leaves much to be desired. Relatively little is known with sufficient confidence at the present time to recommend it as the method of choice in preserving urine on prolonged space flights. Extensive laboratory studies are needed to solve the problems of constituent protection specificity and interference with analytical procedures.

Lyophilization

This method has distinct advantages over most other methods insofar as maintaining the chemical integrity of urine constituents over long storage periods (57), (58), (59), (60). Water is sublimed from urine sample in the frozen state under vacuum. Allegedly, little or no constituent decomposition occurs spontaneously or through microbial action during the frozen state; the lyophilisate, or dried residue, is stable in sealed containers for relatively long periods of time at ambient temperatures (61).

In recent laboratory study to determine the engineering problems associated with freeze-drying, the stabilities of calcium, urea, 17-hydroxycorticosteroids, and catecholamines were shown to be excellent with this method (57). Unfortunately, no data were given to indicate the time of lyophilisate storage prior to analyses.

Personal communications with Hyland Laboratories, which specialize in large volume urine lyophilization on a commercial basis, have revealed important data concerning lyophilisate

stability and constituent integrity (62). Lyophilisates have been analyzed after months of storage, and degradation of important constituents such as the catecholamines, chorionic gonadotropin, 17-hydroxycorticosteroids, pregnandiol, creatine, creatinine, and urea has not been detected. Very labile substances such as amylase, porphyrins, and vanilmandelic acid show excellent stability in urine lyophilisates. Efficient storage for five years at $4^{o}C$ ($39.2^{o}F$) or for one year at room temperature is claimed. Personal research with the very labile pituitary gonadotropic hormones confirms the excellence of lyophilization as a method of preservation. Efficient long term preservation of blood serum constituents by lyophilization is well documented (61).

In-flight lyophilization of urine offers the following advantages (51), (56):

a. Large reduction is made in the volume and weight required for storage of equivalent quantities of urine.

b. No further refrigeration of the lyophilisate is required thus reducing power consumption.

c. Ambient temperature could supply part of the power during lyophilization.

d. Space vacuum can be utilized for sublimination.

e. Concentration of the trace constituents in urine would make their analyses more amenable.

f. The method appears to have advantages in preserving cells, casts, and bacteria.

g. Analytical interferences unique to chemical preservation methods are eliminated.

Lyophilization, however, removes ammonia, carbon dioxide, and volatile organics such as acetone from the urine. Lipoproteins are claimed to be destroyed in the process (61). The above appear to be rather minor drawbacks in view of the many advantages. The major disadvantages to lyophilization are related to engineering aspects of the process.

Ion Exchange Techniques

Ion exchange and sorption technologies are well advanced and documentation is readily available (63), (64). Applications to urine constituent isolation and preservation, however, have not been investigated to any great extent. Catecholamines are adsorbed on alumina under basic pH conditions and subsequently eluted by acid (65). Recoveries are good. 17-hydroxycorticosteroids have been successfully adsorbed on charcoal and eluted with ammoniacal ethanol without any apparent alterations in structure. Recoveries are also claimed to be good (66).

Theoretically, the idea of removing urine constituents on ion exchange beds or various other sorbants for preservation and later analyses seems feasible. An extensive laboratory study would have to be completed before the technique could be recommended as a preferred choice for urine preservation. Laboratory studies related to sorption and ion exchange techniques are listed. The following determinations would have to be made and tested:

a. Sorbant materials required to isolate selected urine constituents.

b. Methods for quantitative removal of selected constituents.

c. Effects of adsorption and absorption on constituent chemical integrity.

d. Sorbant and constituent degradation via bacterial action during storage.

e. Adequate post-sorption storage conditions.

Ion exchange or sorption processes with liquid urine under zero gravity conditions, effects of acceleration and vibration on resin beds, and weight-volume restrictions due to materials are among the problems.

Vacuum Distillation (Drying)

Vacuum distillation techniques have been used successfully for hundreds of years to remove solvents from solutes. The procedure has not been used to concentrate urine as a means of preservation. It seems doubtful that the method has any merit other than a reduction in storage volume. Urine degradation would likely occur, particularly if heat were supplied,

during the evaporation procedure and the concentrate or residue would be very susceptable to both chemical and microbial decomposition.

Refrigeration (Chilling)

Reduction in urine temperature to approximately $4^{o}C$ ($39.2^{o}F$) reduces microbial activity and rates of spontaneous chemical decomposition. This technique is often used when urine is to be preserved for only a few days (26), (28), (52). For longer storage periods, it is used as an adjunct to chemical preservations. Refrigeration, as a means of preserving urine on prolonged space flights, is inadequate for many constituents unless combined with chemical methods.

Frozen Storage

Freezing is generally recognized to be a high quality preservation method for biological fluids (26), (56). Frozen storage time data for urine constituents are not available; however, conclusions based upon similar biological materials give one reason to be optimistic about its applicability to urine (61). Blood constituents, viable sperm cells, tissue cultures, and microbes have been preserved for months by rapid freezing and storage at low temperature. It seems doubtful that chemical composition would be altered by freezing since it has been shown that chemical change is largely the result of bacterial multiplication. These assertions are substantiated by freeze-dry storage data (62).

The major concern with freezing appears to be the rate at which it is accomplished (61). Slow freezing, as in a refrigerator, destroys bacteria, casts, and other cellular bodies by rupturing the membranes. This results from the formation of large ice crystals. Rapid freezing, by immersing the sample in a dry ice-alcohol bath or cryogenic fluid such as liquid nitrogen, prevents cell rupture by limiting internal ice crystal size. At the end of the storage period, rapid thawing is required so that small crystals are prevented from growing larger as the sample is brought up to the freezing point. This can be accomplished by immersing the sample in a heated bath. It is at this stage where chemical degradations may occur.

Several alternatives are available for preserving urine casts and cells for microscopic examination when the urine is to be preserved by freezing. They are:

a. Remove the cellular bodies and casts before freezing. This could be done by filtering a small volume of urine (1 or 2 ml) through a filter of known surface area. Formed elements which are retained on the surface of the filter are then fixed, stained, mounted and stored for later microscopy (67). Formed elements could also be filtered or centrifuged from 1 to 2 ml of urine and stored at $4^{o}C$ in an isotonic solution heavily spiked with antibiotics.

   Preliminary removal of formed bodies from urine eliminates the need for rapid freezing of the sample and one is more assured of getting an intact specimen for microscopy. Also eliminated by this procedure is the need for rapid thawing of the urine samples prior to chemical analyses. Thus, the danger of constituent alteration can be avoided.

b. Glucose, glycerol, and dimethyl sulfoxide solutions have been used with varying degrees of success in preserving cellular bodies from slow freezing (61). This technique may be possible with urine so that preliminary removal of these particles will not be necessary. This technique, however, could lead to problems of interference with chemical analyses. This would certainly be true when using glucose as a freeze-protection agent.

c. Rapid freezing without protective agents has given good results with blood in preserving cellular bodies for microscopy (61). Approximately 90 percent of the erythrocytes and leucocytes remain morphologically intact. Storage temperatures varied between $-40^{o}C$ ($-40^{o}F$) and $-90^{o}C$ ($-192^{o}F$) with the best results obtained at the lower temperature. This procedure might give excellent storage results with urine. The engineering aspects in space, however, could be prohibitive.

Combination of Chemical and Other Methods

As in the case of blood, the only apparent useful combination of chemical and another method is chemical plus refrigeration. The use of chemicals in conjunction with refrigeration for urine preservation might very well increase the duration of stability of some components over that achieved with chemicals or refrigeration alone. (See Chemical and Refrigeration above). Little information is available concerning this approach, however. Whether periods of stability of constituents long enough for resupply periods of 45 days or more could be attained would have to be determined by laboratory study.

Conclusions

From the standpoint of the analyst, the best urine preservation method will (1) preserve the greatest number of selected urine constituents, and (2) not interfere with analytical procedures. On this basis, lyophilization is the method of choice. In the event of prohibitive engineering restrictions, preservation by freezing and chemical preservation can be considered second and third choices, respectively.

The following outline compares these three preservation methods as related to other factors influencing suitability of preservation method.

Storage Time Efficiency

a. Chemical

Storage time is variable depending upon chemical agent or agents used. If preservatives prohibit microbial growth only, then some spontaneous hydrolysis, oxidation, or reduction can occur over long storage periods. Use of refrigeration, anti-oxidants, and acidulants can increase storage time stability. In general, stable storage times may vary from a few days to a month or more.

b. Lyophilization

Excellent. Labile peptides, proteins, and hormones have been preserved for years at $4^{o}C$ ($39.2^{o}F$) to $8^{o}C$ ($46.4^{o}F$) and for shorter periods at ambient temperatures.

c. Frozen Storage

No long term storage data are available but based upon frozen storage data for other biological fluids, this method may be excellent for urine also. Storage efficiency may be second only to that of lyophilization.

Sample Volume Required

The urine volume required for analysis is independent of the preservation method. Urine volume requirements are the same for the three methods.

Complexity of Sample Preparation for Preservation

a. Chemical

Sterile evacuated storage containers could be precharged with the correct amount of chemical preservative. Some manual mixing action will be required to facilitate blending of the sample and the preservative.

b. Lyophilization

Foaming during the drying phase may require samples to be treated with a protein precipitant. Each lyophilization chamber could thus be charged with sufficient precipitant in advance. However, a separate aliquot for protein determination would then be required.

c. Frozen Storage

No problems with sample preparation are expected if the specimen is to be solidified by ordinary refrigeration. If freezing is to be accomplished by evaporation, then the problem of foaming may be encountered.

Special Biological and Analytical Considerations

a. Chemical

Preservative-induced or spontaneous hydrolysis and oxidation of labile constituents may occur. Precipitates may form with changes in pH. Various preservatives can interfere with analytical procedures.

b. Lyophilization

Protein precipitation may be required to prevent foaming during the drying process if distillation is used to initially freeze the sample. Precipitant may affect subsequent analyses. Volatile components such as ammonia, carbon dioxide, acetone, and other organics are lost. Rapid freezing or freeze protection agents are required to preserve casts and cellular bodies.

c. Frozen Storage

Precipitates usually form during freezing and require special attention after thawing. Rapid freezing or freeze-protection agents are required to preserve casts and other formed bodies.

Complications from Common Ions

This consideration applies to chemical preservation only. Preservative agents such as sodium fluoride, sodium bisulfite, hydrochloric acid, sodium borate, and sulfuric acid prevent analysis for chloride, sulfate, and sodium.

5.4.3 SWEAT AND FECES

Preservation of sweat and feces for post-flight analyses of sodium, potassium calcium and chlorides is relatively simple; because only elemental analyses are involved, no problems arise from the lability of the constituent sought during temperature variations, oxidation, bacterial attack or chemical decomposition of the sample.

The simplest and most economical technique for preserving sweat and feces is chemical. Compounds containing sodium, potassium, calcium or chloride must be avoided in order to obtain accurate measures of these elements in collected samples. Furthermore, the preservatives chosen should not interfere with the assay of the indicated electrolytes. A combination of propylene glycol, orthophenylphenol and 4 chloro-2-phenylphenol, 6 chloro-2-phenylphenol and Gentian violet, reported to have been used on the Gemini missions, would interfere with chloride determinations, but a mixture of a phenol and a quarternary ammonium compound should be considered. Iodine and bromine compounds may be suitable, but since the analytical method chosen is apparently not specific for chloride, the presence of other halogens could affect the results.

The problems of toxicity of chemical preservatives and of handling them in zero gravity are similar to those for blood and urine. For pertinent comments, see Paragraphs 5.4.1.2 and 5.4.2.

5.5 TRADE-OFF CONSIDERATIONS OF BIOLOGICAL REQUIREMENTS VERSUS ENGINEERING PENALTIES

In the course of this study, it was discovered that the limitations of Appendix B could be met by the engineering requirements for most types of preservation considered. For this reason, it was decided to choose whatever method was the best from the biological point of view for preserving specimens especially since some variables are particularly sensitive to alteration by the choice of non-optimum preservation techniques. In most cases, in fact, there is only one choice which can be made. In addition, it becomes apparent that some analyses could not be done on material preserved in any fashion and must be done on-board if at all.

In other words, biological requirements did not impose engineering penalties which were not within the allowances made for power, weight, and volume in Appendix B, "Preliminary Design Features" with the exception of low temperature technique for blood cells. Since biological data of the finest quality is desired, it is not considered worthwhile to try to reduce engineering penalties, which might compromise biological quality, especially since these penalties are well within the limits of "Design Features".

The general criteria which were employed in evaluating and selecting preservation techniques are listed in Table 5-8 in the order of their importance.

Table 5-8. Criteria for Evaluation and Selection of Preservation Techniques

CRITERION	PRIORITY
Stability of Constituents	1
Accuracy of Subsequent Analysis	2
Safety of Astronauts	3
Weight, Power and Volume Penalties	4
Complexity of Procedure (Ease of performance)	5
Complexity of Equipment	6

5.6 RATING OF PRESERVATION METHODS

A tabulation of ratings for preservation methods is given in Table 5-9 on Several bases of importance.

5.6.1 RATING OF PRESERVATION METHODS ON THE BASIS OF CONSTITUENT STABILITY

It is very difficult to find experimental bases for rating the preservation methods discussed in this report on the basis of constituent stability. Very little information is avilable in the way of experimental data. Various groups (e.g. Lovelace Foundation) and individuals, however, by means of judgment based upon extrapolation from what little experimental data there is and a logical approach based on general knowledge, have tried to establish the optimum and permissible preservation techniques. In some cases, these opinions are little more than hunches, and in all cases they require experimental verification by extensive laboratory efforts.

We will limit the discussion in this section to the stability of chemical constituents of blood, urine, feces and sweat including proteins, enzymes and hormones. The almost insurmountable difficulties in preserving formed elements has been referred to elsewhere. During the survey conducted of key references on the problem of stability, no information was found concerning the effect of sample size with one exception. (Small aliquots (0.1 - 0.2 ml) of plasma rapidly lose prothrombin activity even when refrigerated (70)).

Table 5-10 gives the duration of stability of various constituents of blood, plasma or serum at various temperatures based on experimental analyses. This is a summary of the information available in the references cited. Other references may give opinions concerning duration of stability under various conditions, but these are usually not backed up with experimental data. For example, lyophilization is generally believed to be an ideal method for preservation of most chemical constituents of blood, but this belief has, to our knowledge, not been put to the test by extensive laboratory investigation and analysis. What is recorded in Table 5-10 can be relied upon as data rather than opinion. In a few cases, different investigatiors have obtained differing laboratory results. These are honest contradictions in experimental data rather than differences of opinion.

Table 5-9. Ratings of Preservation Techniques

	CHEMICAL	REFRIGERATION	FREEZING	VACUUM DIST.	LYOPHILIZATION
SAFETY	5	10	8	2	1
COMPLEXITY	10	5	4	2	2
EASE OF PERFORMANCE	5	10	9	2	1
STABILITY OF CONSTITUENTS					
(a) Blood	2	3	8	3	7
(b) Urine	4	3	8	2	9
(c) Sweat	8	5	10	6	10
(d) Feces	8	5	10	6	10
(e) Microorganisms	1	6	3	1	8

10 = Best

1 = Worst

Table 5-10. Duration of Stable Storage for Blood, Serum and Plasma Constituents

	ROOM TEMPERATURE	REFRIGERATION	FREEZING	REFERENCES AND COMMENTS
CREATINE	5 days with fluoride or fluoride and thymol	24 hours	at least 6 months	50, 26 - Ratio between creatinine and creatine changes unless frozen due to pH dependent equilibria
CREATININE	5 days with fluoride or fluoride and thymol	24 hours	at least 6 months	26, 50
SERUM PROTEINS	3 days	1 month	at least 6 months	26, 50, 70 - Patient must be in basal (resting and fasting) state at time of withdrawal of samples to avoid spurious results
MUCOPROTEINS	2-7 days at 30°C			26
SODIUM	at least 2 weeks	at least 2 weeks		26
	at least 8 hours	24 hours	at least 1 year	70 - Longer storage at room temp. or at 4°C may result in protein precipitation which may alter precise measurement. Hemolysis should be avoided and serum should be quickly separated from clot. Patients may have to be in basal state.
POTASSIUM	at least 2 weeks	at least 2 weeks		26
	at least 8 hours	24 hours	at least 1 year	70 - See comment for Sodium
CHLORIDES	1 week	1 week		26
	at least 8 hours	24 hours	at least 1 year	50, 70 - See comment for Sodium
PHOSPHATES		1 week		26 - Serum or plasma must be separated from cells for stability
	at least 8 hours	24 hours	at least 1 year	70 - See comment for Sodium

Table 5-10. Duration of Stable Storage for Blood, Serum and Plasma Constituents (Cont.)

	ROOM TEMPERATURE	REFRIGERATION	FREEZING	REFERENCES AND COMMENTS
ALKALINE PHOSPHATASE	8 hours	8 hours	at least 1 week	70
			less than 6 months	50 - Plasma
			16 months	26 - Serum
CALCIUM	at least 8 hours	24 hours	at least 1 year	26, 70 - See comment for sodium
MAGNESIUM	1 week			26
	at least 8 hours	24 hours	at least 1 year	70 - See comment for sodium
MANGANESE				
ZINC				
BICARBONATE	Stable until bacterial decomposition begins			26 - pH of sample must be known at time of withdrawal of sample
SULFATES	4 days	4 days	Frozen samples are more stable, but no time limit given	26
NPN	About 1 day About 5 days with fluoride or fluoride and thymol	at least 1 week	less than 6 months	26, 50, 70
BUN	About 1 day	at least 1 week	at least 6 months	26, 50, 70
	About 5 days with fluoride or fluoride and thymol			
URIC ACID	10-14 days with fluoride or fluoride and thymol	3 to 5 days at least 1 week	at least 6 months	26 50, 70

Table 5-10. Duration of Stable Storage for Blood, Serum and Plasma Constituents (Cont.)

	ROOM TEMPERATURE	REFRIGERATION	FREEZING	REFERENCES AND COMMENTS
GLUCOSE	Unstable	Unstable	Less than 6 months	26, 50 - A great deal of controversy exists concerning stability of glucose
	1 week with fluoride or fluoride and thymol			
LIPIDS	Unstable	Unstable	Recommen. but stable duration unknown	26, 70 - Situational stress, even that of blood withdrawal tends to alter blood lipids significantly
AMINO NITROGEN	Unstable	Unstable	"Lone periods of time"	26
LACTIC ACID	Unstable	Unstable Trichloracetic acid filtrates stable at least 3 days	Unstable	26
BILIRUBIN	8 hours			70
	about 2 days	4 to 7 days	3 to 6 months	26, 50 - Must store in dark (Light sensititive). Hemolysis must be avoided
PBI	at least 5 weeks			26
CATECHOLA-MINE				
THYROXINE	At least 11 days at 30°C			26
TBPA				
ADH				
ACTH				
HEMOGLOBIN	At least 1 week at 30°C			26

Table 5-10. Duration of Stable Storage for Blood, Serum and Plasma Constituents (Cont.)

	ROOM TEMPERATURE	REFRIGERATION	FREEZING	REFERENCES AND COMMENTS
FIBRINOGEN	7 days	4 weeks		26
FIBRINOLYTIC ACTIVITY				
PROTHROMBIN ACTIVITY	At least 18 hours			70
PTC				
AHG				
IMMUNO GLOBULINS				
LDH ISOZYMES	At least 8 hours	at least 1 week	at least 1 month	70 - Changes in the quantitative distribution of isozymes may occur. Losses of enzyme activity in abnormal sera may be extremely variable, and may not follow any pattern. Patient may have to be in basal state because enzyme levels may otherwise be affected.
IMMUNE BODIES	Merthiolate preserves for unknown length of time	Refrigeration and merthiolate preserve for unknown length of time	Indefinite	28
TRANS-FERRINS	4 days	at least 1 week	Many months	26
METHEMO-GLOBIN	Unstable	Unstable		26

The temperatures of storage for Table 5-10 are as follows: Room Temperature 20° to 25°C; refrigeration 4°C ± 2°C; freezing -10 to -12° C. Sometimes the data do not specify the temperature of frozen storage, but usually it refers to storage in the freezing compartment of a refrigerator which may be as low as -17 to -18°C. Winsten (70), however, insists on a minimum freezing temperature of -12°C, but he does not give a rational for this limit. We are of the opinion that the lower the storage temperature in the frozen state, the better the degree of preservation. We also believe that a great deal more stability can be achieved if the temperature of frozen storage is below the eutectic point (-28°C for serum). These opinions would have to receive experimental verification, however.

Frazer (71) of the Lovelace Foundation has tried to combine experimental data and scientific judgment to determine the duration of stability using the following types of storage for most of the constituents of interest in blood, urine, sweat and feces: Unprocessed, preservative, refrigeration (<5°C), preservative and refrigeration, lyophilization and freezing (-75 to -100°C). He has summarized his opinions in Tables III and IV of that report. But, as Frazer states, "It is emphasized that by no means all of the durations quoted have been validated experimentally, and it is recommended that tests be conducted to determine the reliability of the storage," (p. 14) and again, "...it is again emphasized that the storage techniques discussed have not been validated in all cases. It is recommended that the various techniques and durations of stable storage be experimenatally tested under realistic conditions." (p. 26)

An actual rating scheme for various techniques of preservation of specific constituents is presented in Table 5-11. The basis of the ratings is stability of storage. The ratings vary from 1 (least stable) to 10 (most stable). It must be emphasized, however, that these ratings are based upon scientific judgment for the most part and upon experimental evidence in those few cases where this has been available. We must thus point out, as did Frazer, that the reliability of preservation for stability of constituents must await laboratory validation.

Table 5-11. Ratings of Preservation Methods on the Basis of Stability of Constituents

Blood, Plasma, or Serum	Chemical	Refrigeration	Freezing	Vacuum Dist.	Lyophilization
Creatine	3	2	10	5	10
Creatinine	3	2	10	5	10
Serum Proteins	1	4	10	2	10
Mucoproteins	1	2	8	2	8
Sodium	5	3	10	10	10
Potassium	5	3	10	10	10
Chlorides	5	3	10	10	10
Phosphates	1	2	10	10	10
Alkaline Phosphatase	1	1	7	3	5
Calcium	5	3	10	10	10
Magnesium	5	3	10	10	10
Manganese	5	3	10	10	10
Bicarbonate	5	3	10	5	10
Zinc	5	3	10	10	10
Sulfates	1	2	10	10	10
NPN	3	5	7	2	2
BUN	3	5	10	10	10
Uric Acid	6	5	10	10	10
Glucose	5	1	7	6	7
Lipids	2	1	7	3	7
Amino Nitrogen	1	1	8	4	8
Blood Lactic Acid	1	2*	7*	1	1
Bilirubin	1	4	9	5	9
PBI	1	3	10	4	10
Catecholamines	1	2	8	4	8
Thyroxine	1	3	9	5	9
TBPA	1	2	8	3	8
ADH	1	2	8	3	8

Table 5-11. Ratings of Preservation Methods on the Basis of Stability of Constituents (Cont.)

Blood, Plasma or Serum	Chemical	Refrigeration	Freezing	Vacuum Dist.	Lyophilization
ACTH	1	2	8	3	8
Hemoglobin	1	2	9	3	7
Fibrinogen	1	6	10	4	10
Fibrinolytic Activity	1	2	8	3	8
Prothrombin Activity	1	2	8	3	8
PTC	1	2	8	3	8
AHG	1	2	8	3	8
Immunoglobulins	1	2	8	3	8
LDH Isozymes	1	3	8	2	7
Immune Bodies	5	6+	10	4	10
Transferrins	1	4	8	3	8
Methemoglobin	1	1	9	3	9
Urine					
Creatine	7	2	10	2	10
Creatinine	7	2	10	2	10
Mucoproteins	1	2	8	2	8
Sodium	5	3	10	10	10
Potassium	5	3	10	10	10
Chlorides	5	3	10	10	10
Calcium	2	3	10	10	10
Magnesium	5	3	10	10	10
Manganese	5	3	10	10	10
Zinc	5	3	10	10	10
Sulfates	3	2	10	10	10
Total Nitrogen	3	5	7	1	1
Glucose	6	1	7	6	7
Acetone Bodies	2	3	8	5	6
Albumin	1	2	9	3	9
17-Hydroxycorticosteroids	7	3	10	1	10
Aldosterone	1	2	8	3	8

Table 5-11. Ratings of Preservation Methods on the Basis of Stability of Constituents (Cont.)

Urine	Chemical	Refrigeration	Freezing	Vacuum Dist.	Lyophilization
Serotonin	7	3	9	5	9
Proteins	1	3	8	3	8
Feces and Sweat					
Sodium	8	5	10	6	10
Potassium	8	5	10	6	10
Chlorides	8	5	10	6	10
Calcium	8	5	10	6	10

* Requires acid filtrate

\+ Requires chemical preservatives as well

1 = Least Stable

10 = Most Stable

5.6.2 RATING OF PRESERVATION TECHNIQUES IN TERMS OF SAFETY AND EASE OF PERFORMANCE OF PROCEDURES FOR ASTRONAUTS

Chemical

Safety - Most chemical preservatives which might be used are very toxic to man. If the suggested approach of storing chemical preservatives in the sample container is used, great care must be taken in engineering these containers so that there is no chance of spilling the preservatives into the cabin atmosphere where they could prove irritating or even dangerous to crew members. A possible approach is flexible plastic containers having preservative in a sealed compartment which could be opened after sample is introduced by means of a manually operated seal. Even with this method, however, the safety problem still exits.

Safety Rating: Moderate.

Ease of Performance - Depending on the design of sample containers, chemical preservation could be quite difficult in getting samples into containers without venting preservatives into the cabin. With adequate training, however, crew members could learn to do this with relative facility and ease. In addition, compartmented containers with manually operated seals between compartments would make this technique even easier.

Ease of Performance: Moderate.

Refrigeration

Safety - The approach suggested for refrigeration, namely the use of a space heat sink should be very safe. The only dangers involved would be the release of coolant to cabin atmosphere due to mechanical breakdown of the coolant pump or coolant lines. It would be necessary to insure against such an event in the design and construction of flight hardware. Rigid testing procedures would assure high reliability of the system.

Safety Rating: High

Ease of Performance - Refrigeration is extremely easy to perform on the part of the astronaut. It involves simply placing containers which may require prior processing of various types (see other sections) in the refrigeration unit.

Ease of Performance Rating: High.

Freezing

Safety - The approach suggested, namely a Freon vapor cycle unit, would have to be engineered for adequate safety of the astronauts in space flight. Since these units are still in the developmental stage for use in a zero gravity environment, appraisals of safety would have to await flight testing. However, considering the rigid testing procedures that such units

would have to pass to be considered as flight hardware, the final level of safety would be very high.

Safety Rating: High

Ease of Performance - If a "cold finger" approach to initial freezing is used, it may be necessary for the astronaut to freeze a sample in a "cold finger" module, and then transfer to a frozen storage module. This is not a very complicated task, however. As in refrigeration, there would be preliminary manipulations of the sample which are described elsewhere. But the actual freezing process itself would be quite easy.

Ease of Performance Rating: High.

Vacuum Distillation

Safety - The approach suggested for vacuum distillation employs space vacuum via hand operated valves on special containers. This would seem to be a relatively dangerous procedure with the possibility of error in the manipulation of valves leading to significant loss of cabin atmosphere. Of course, with adequate training, the astronauts would not be likely to make such an error, but the possibility of manual error or sticking of valves makes this technique more risky than others.

Safety Rating: Low.

Ease of Performance - The operation of the vacuum distillation apparatus would require a good deal of operator finesse. The sample would have to be introduced into the chamber, the chamber sealed, valves positioned correctly, chamber connected to space vacuum and chamber degassed for predetermined lengths of time at two pressures. For these reasons, this procedure would be considered relatively difficult.

Ease of Performance Rating: Low.

Lyophilization

Safety - Since the approach to lyophilization is similar to that for vacuum distillation, the same comments apply. In addition, the container will become quite cold during this process, permitting the possibilities of valves sticking and astronauts' "burning" themselves on the cold container.

Safety Rating: Low.

Ease of Performance - Due to the similarity of this approach to that of vacuum distillation, the same comments apply. The difficulty of operation is even somewhat greater because the length of time that the container must be attached to space vacuum would be longer.

Ease of Performance: Low.

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SECTION 6

COLLECTION AND HANDLING OF SAMPLES

6.1 COLLECTION

The collection task involves the interfaces with the men, collection and sampling devices, and certain of the processing required prior to preservation. Proper labeling of the collected samples is of prime concern. An automated means to stamp the subjects' name, time, date, etc. on each sample container will assure proper identification when the samples are analyzed.

6.1.1 FECES SAMPLING

A normal stool for a man on a space type diet will probably weight about 150 grams. Approximately 10 to 12 grams of excreted feces is required for a sample to determine dry weight, sodium, potassium, chlorides, and calcium in duplicate; however, the stool is not homogenous because of chemical and bacterial stratification. The stratification necessitates:

a. The taking of the entire stool as the sample,

b. Homogenizing the stool before sampling, or

c. Mixing the stool with water and taking an equivalent sample.

The above order is also the order of increasing system complexity.

The present "state-of-the-art" for feces waste management is the Apollo system. Upon excretion, the stool is transported to a porous bag by an air flow. The stool is retained in the bag and the air passes thru to the environmental control system. A germicide (see section on Chemical Preservation) is added to the stool and the porous bag is sealed in a nonporous plastic bag and stored. It would be a very simple matter to set aside feces

samples with this system. An alternate approach has been developed by GE (1) which for normal use shreds the stool and spreads it on the inside wall of a storage container where it is vacuum dried. A sample stool may then be collected in a porous bag, similar to the Apollo System, and preserved by the selected preservation technique.

Homogenizing the stools before sampling for chemical analyses is not esthetically pleasing if done manually (kneading in plastic bag) or quite complex and messy if done mechanically. This method is thus deleted from further consideration.

Systems have been developed by GE (2 and 3) for mixing and blending the stool with water while cleansing the user's anal area with warmed flush water. (See Figures 6-1, 6-2, and 6-3.)

Blended feces from men on a space type diet showed particle sizes to be smaller than 0.01 inch (0.12 mm).

This system obviates the need for toilet tissue and is thus much more sanitary. Once a fecal slurry has been produced, a homogenized sample of the slurry is taken for analyses and the remaining slurry is processed to recover the flush water. The sample is taken with a hypodermic syringe introduced into the fecal blending section. If, nominally, 600 ml of flush water are mixed with 150 grams of feces, then a 50 ml sample is taken to give 10 grams of feces for analysis. The samples may be preserved by any of the preservation techniques.

The latter feces sampling technique is recommended because of its adaptability to the more sophisticated waste management system and because it is sanitary, simple to use, and offers an opportunity to determine the amount of feces excreted without collecting the entire stool. This is accomplished during the analysis by comparing the amount of wet solid to the total sample weight. For example, with the above mixtures, 10 grams of feces in the 50 ml sample indicates a 150 gram stool.

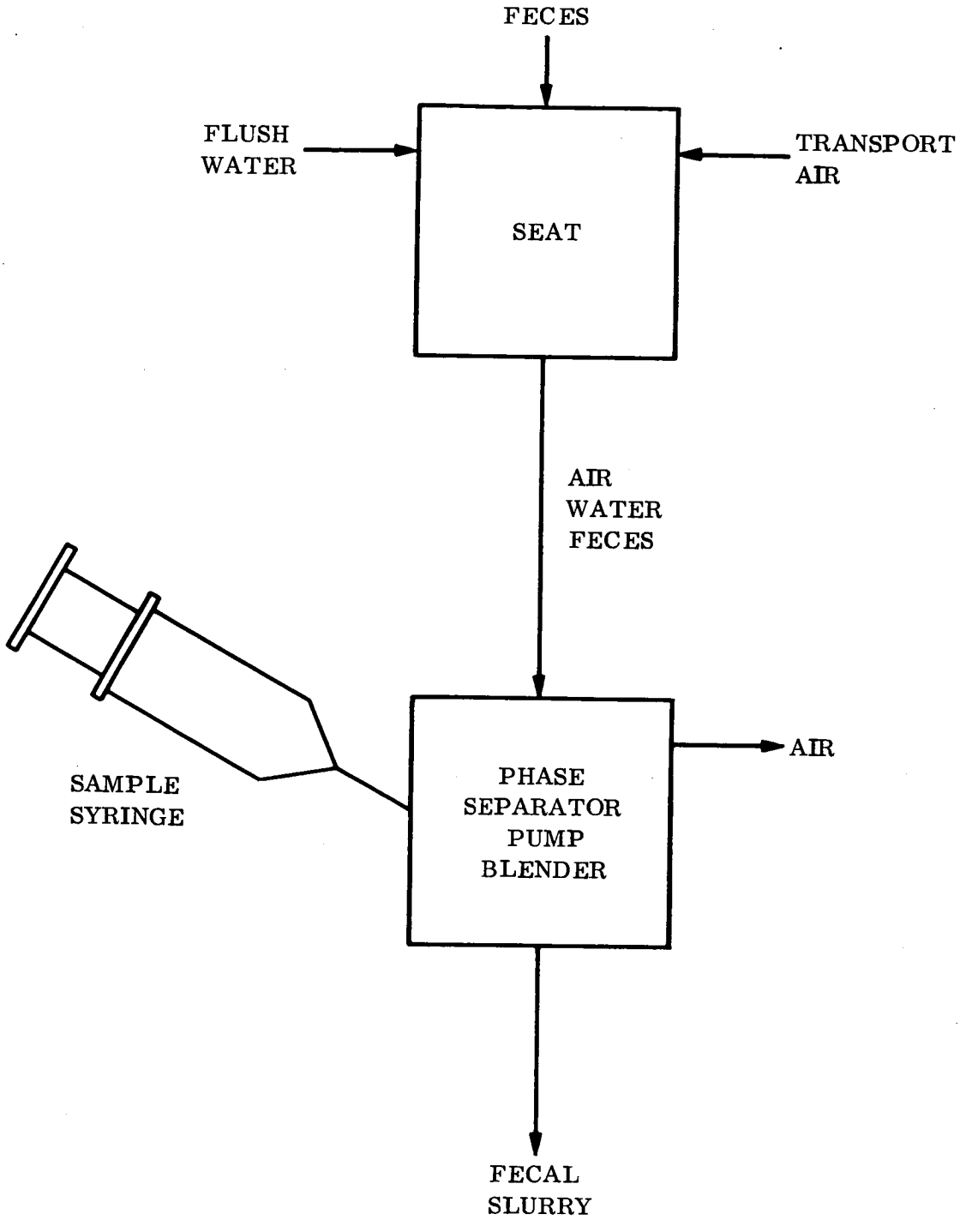

Figure 6-1. SemiAutomatic Feces Sampling Unit

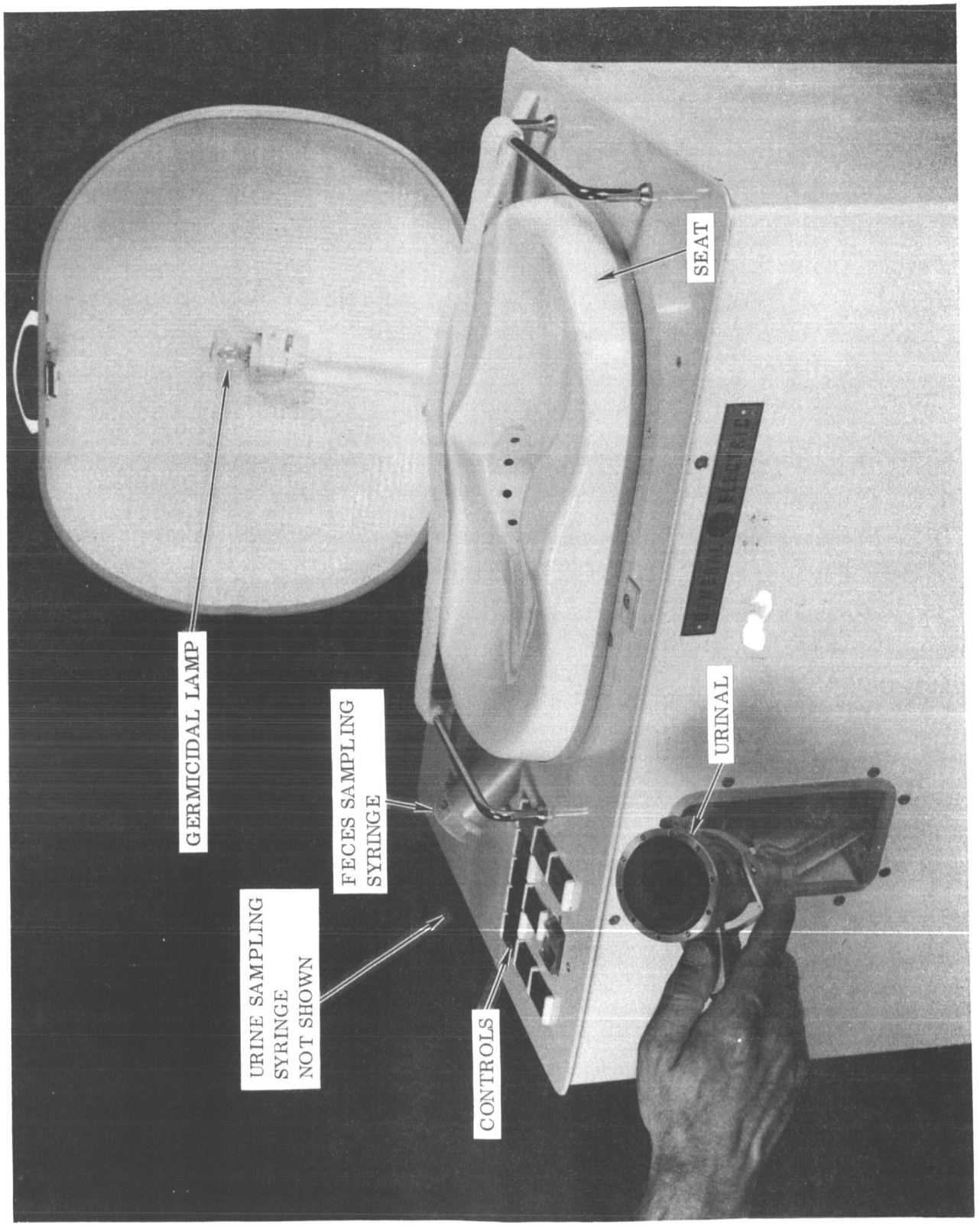

Figure 6-2. Feces and Urine Collection and SemiAutomatic Sampling Assembly

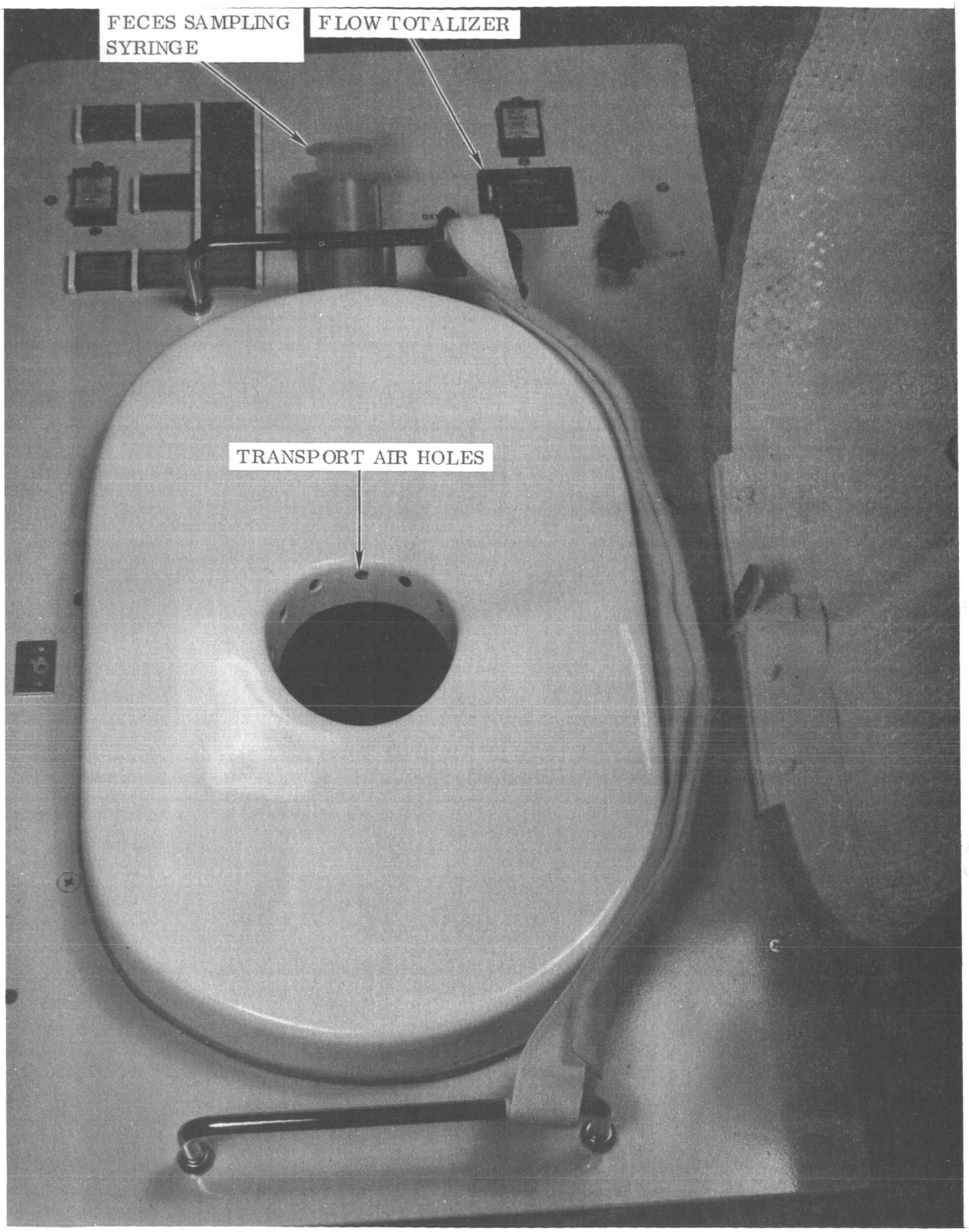

Figure 6-3. Plan View-Feces and Urine Collection and Sampling Assembly

6.1.2 URINE SAMPLING

The present "state-of-the-art" for urine waste management is the Apollo system. Upon micturation, the urine is transported by an air flow to a sponge filled bag where the urine is collected and the air passes thru to the environmental control system. Upon collection, the bag is subjected to space vacuum and the urine is squeezed from the sponge and jettisoned. No flight system is available for urine sampling.

An adult male micturates approximately 6 times a day with a total urine output of 1500 ml per day. Two systems have been developed by GE for semiautomatic and automatic collection of urine samples (2 and 4) and one manual approach is in the design concept stage. Both developed systems utilize an air flow to convey the urine from the urinal to a phase separator. Urine is expelled into a urinal constructed of material impregnated with an antibacterial agent. The urinal has an adjustable membrane which seals around the penis thus preventing leakage during zero gravity operation.

In order to avoid splashing or spilling, the urinal is designed with:

a. Low surface energy material (antibacterial vinyl) on the internal contact surfaces to limit adhesion.

b. A tapered funnel shaped receiver to minimize splashing within the urinal, the exit orifice being directly in line with expelled urine.

c. An air transport medium which enters at the top of the urinal and conveys the urine to the phase separator.

d. A penis seal which prevents urine bubbles or droplets from escaping into the cabin atomsphere.

Without a positive penis seal, small weightless droplets escape to the cabin from the urinal when urine is expelled at high velocities. This is more critical at lower cabin ambient pressures. Splashing is caused when the urinal cohesive and adhesive forces on the urine are overcome by the momentum of the urine. Any resultant escape of an

appreciable amount of urine into the cabin atmosphere would probably be irritating to the crews' eyes and could enhance the growth of microbial contaminants in the cabin. The design features, as listed herein, of the urinal positively prevent urine droplets from entering the cabin atmosphere. This urinal concept is similar to the urinal designed and tested in several GE programs. (See Figure 6-4 and 6-5.) The diaphragm assembly is easily removable so that each man may have his own personal assembly or the seal itself may be replaced after each use. Several fourteen-day tests have been conducted with groups of men using the same assembly. No problems were indicated by the users.

The urine is carried by the transport air and by the urine's initial velocity from the urinal into the phase separator. The phase separator utilizes the mass difference between the transport air and the urine liquid for centrifugal separation with the result that the liquid fills an annular envelope vortex at the outside diameter and the air is drawn from the center of the phase separator. The centrifugal forces are created by a motor driven rotating impeller.

The phase separator centrifugally separates the transport air from the urine and pumps the urine to storage, further processing or space jettisoning.

The semiautomatic sampling system utilizes a hypodermic syringe which is introduced into the phase separator (Figures 6-2 and 6-6). A sample is then manually removed from the collected urine. The automatic system utilizes a dual loop peristaltic pump which has a 10:1 pumping ratio between the loops. The dual loop pump removes the urine from the phase separator with the small capacity loop filling a sample container and the large capacity loop pumping the urine to storage, etc. (Figures 6-7 and 6-8). Both systems utilize methods to measure the total urine volume by either/liquid flowmeters or counting the revolutions of the peristaltic pump. Also, the automatic system with peristaltic pump permits a cross-check of total urine output by measuring the sample volume at the time of analysis, since the sample is 1/10 of the total (other fractions may be used). Sequential

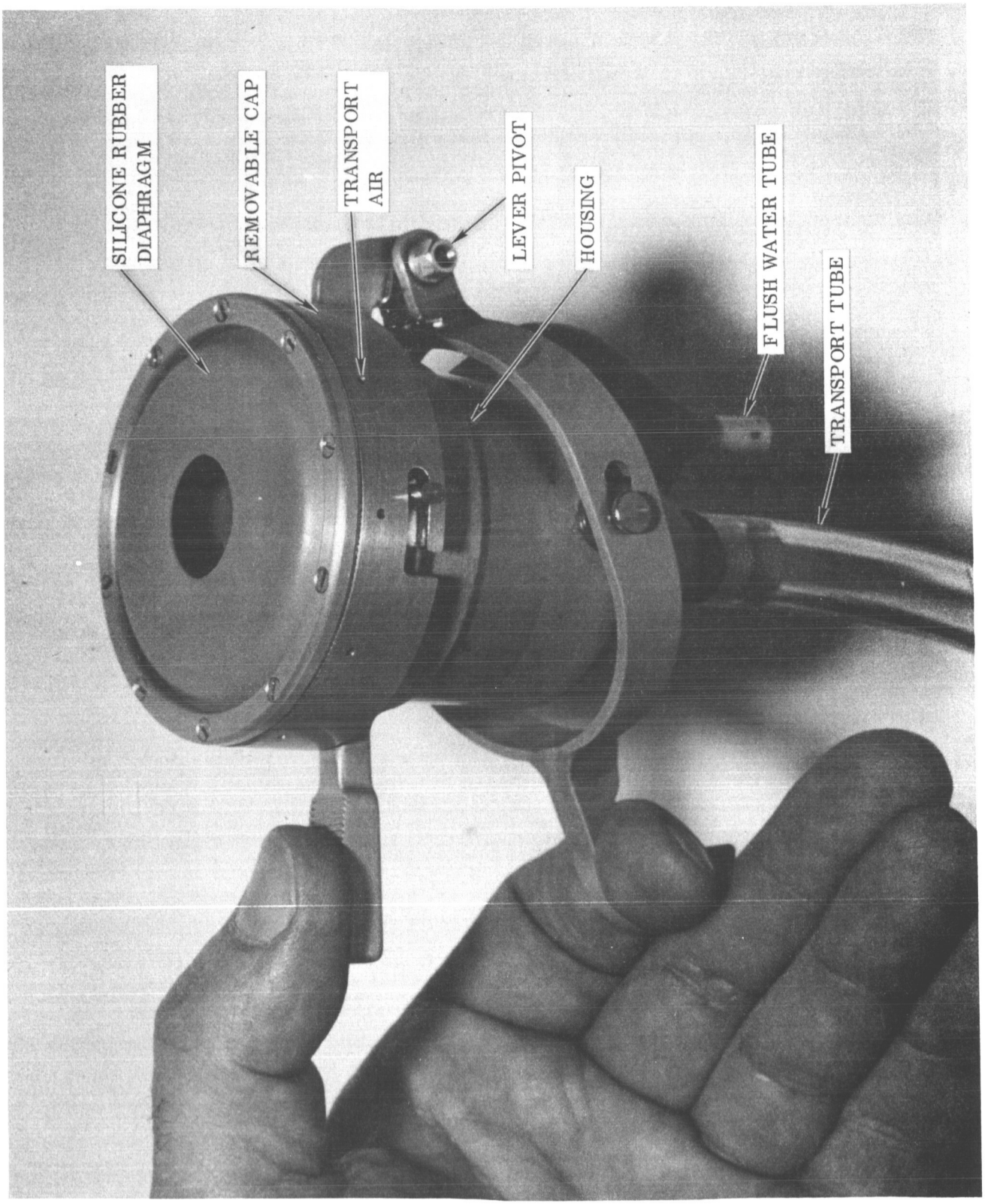

Figure 6-4. Urinal-Normal Position

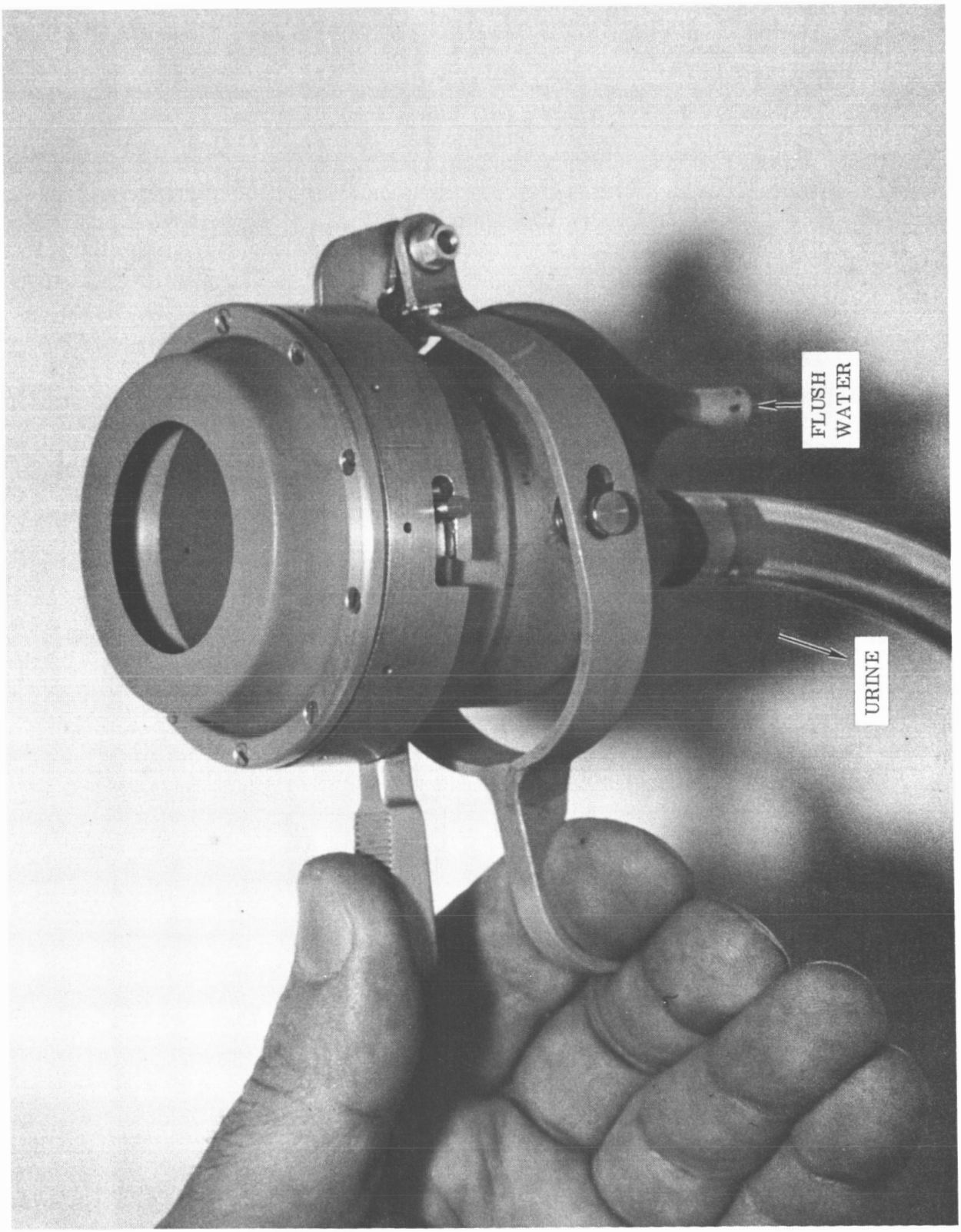

Figure 6-5. Urinal-Expanded Position

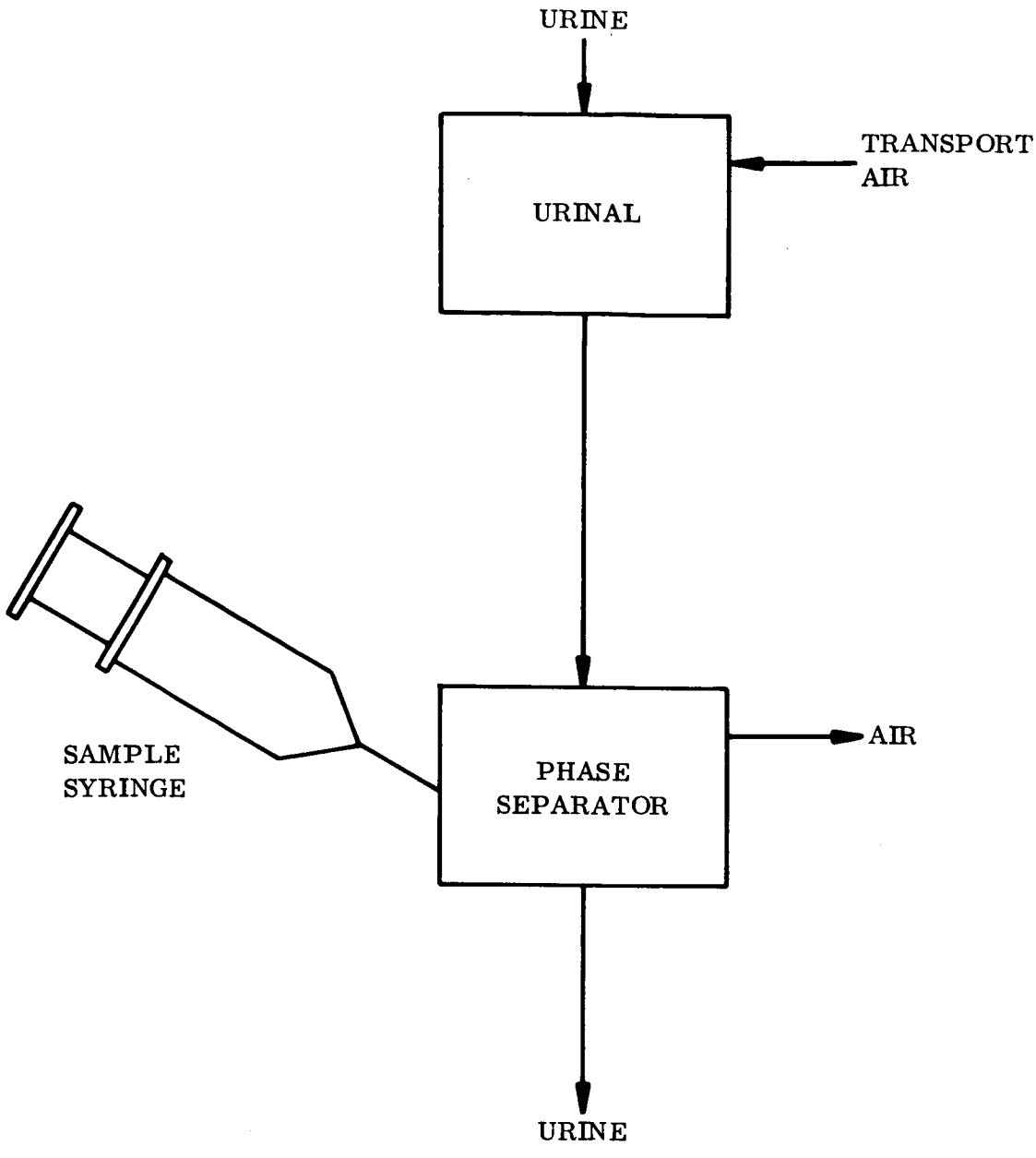

Figure 6-6. SemiAutomatic Urine Sampling Unit

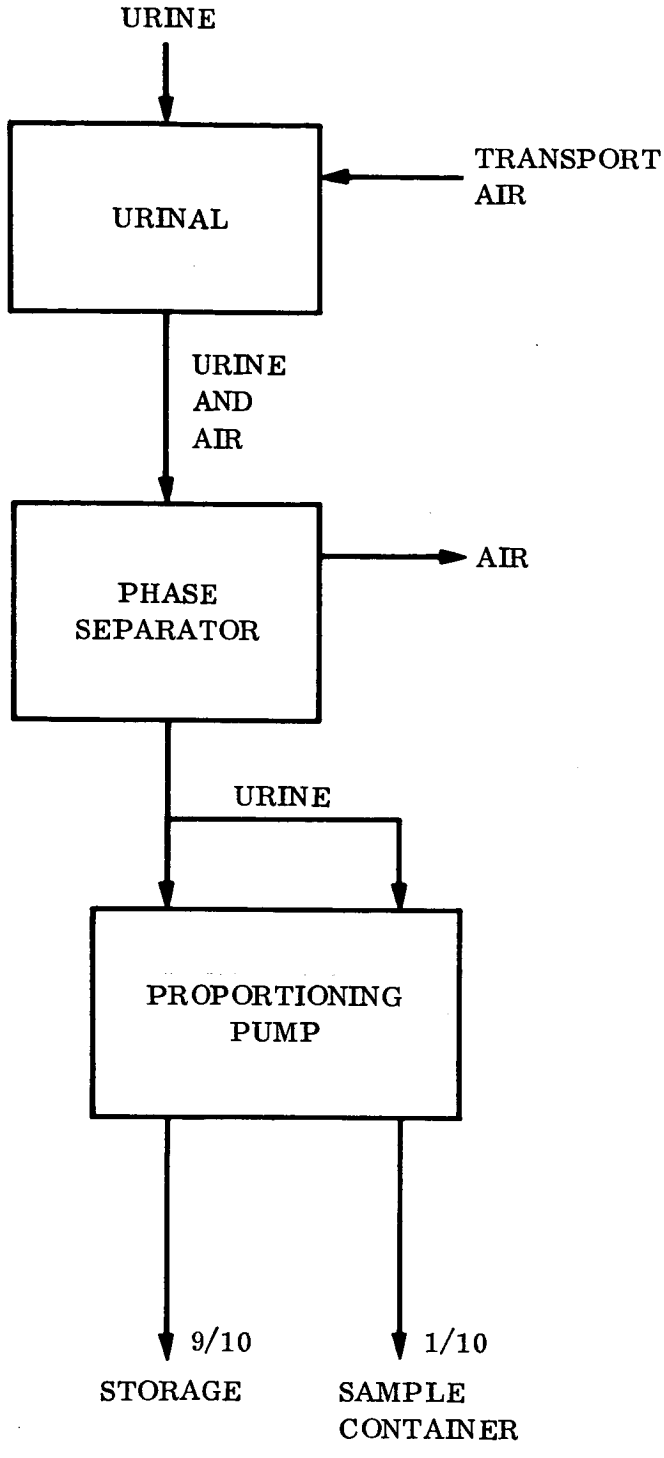

Figure 6-7. Automatic Urine Sampling Unit

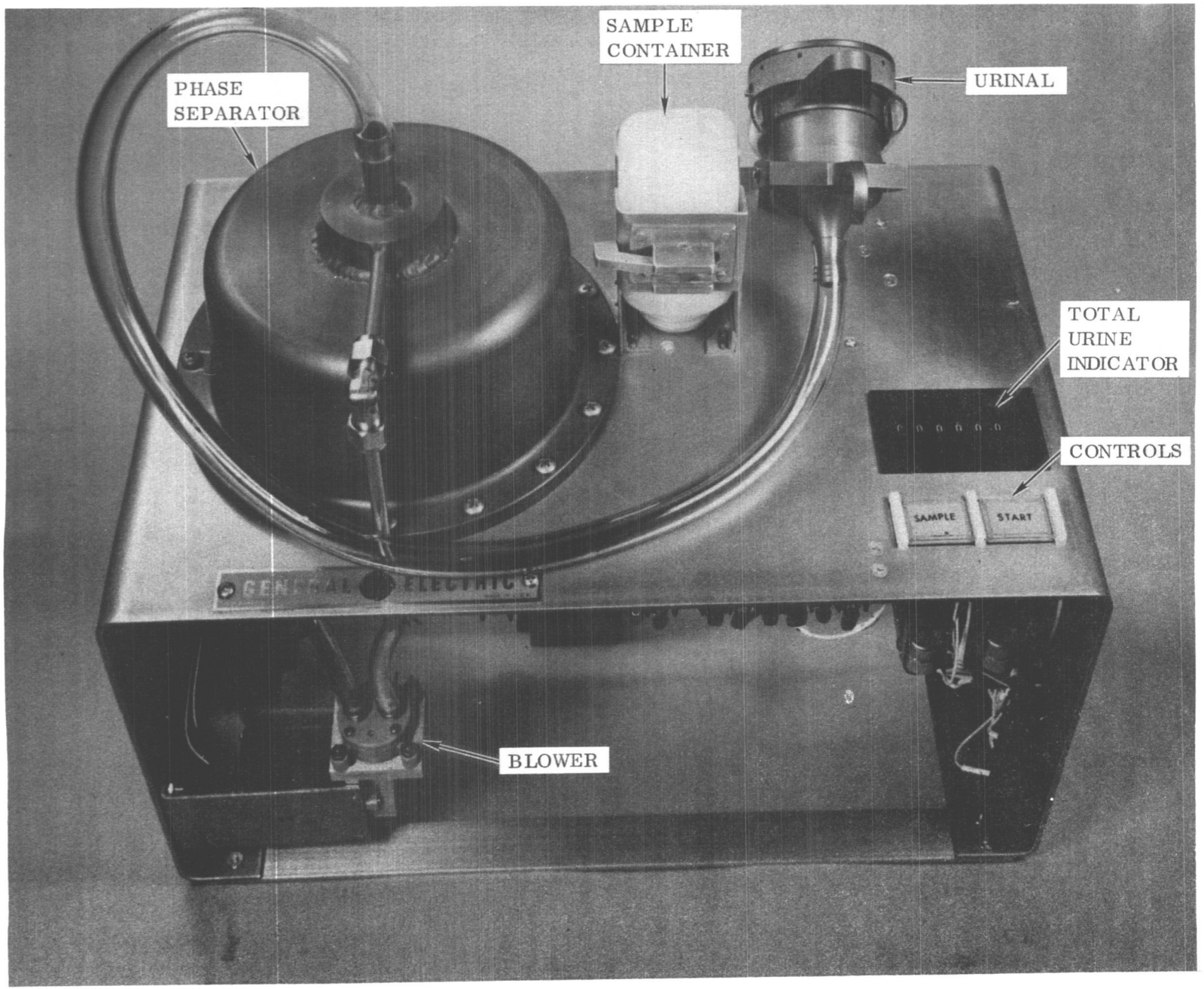

Figure 6-8. Automatic Urine Sampling System

samples may be added to the same sample container to give an integrated sample for several micturations. Either system may be purged with air flush water to assure sample purity.

A manual technique for urine sampling requires urination into a collapsed rubber bag which is sealed after use by twisting or other methods. (See Figure 6-9.) Litmus paper is used to check the pH of the drop remaining on the meatus. The urine bag has a septum at one end which is used to connect the filled urine bag to a filter and a sample bag. The urine bag is squeezed to force the urine through the filter and into the urine sample bag. The solids in the filter and the liquid sample are then preserved and stored for analysis at a later date.

The manual sampling technique will require highly motivated users to routinely collect urine samples. This is a rather odious task and is not likely to be completely successful over a long mission. The automatic sampling system is recommended for this mission to assure adequate accumulation of samples with more accuracy than the manual or semi-automatic systems. Also, there is less likelihood of contamination (or mixing of the different subjects) of samples with the automatic system.

The users of the automatic system have only a few simple tasks:

a. Pickup urinal. (Starts system).

b. Urinates.

c. Depresses sample button if required.

   A sampling program can be completely automated. For example, 10% of the urine can be sampled at each urination and accumulated to give a representative daily urine sample. The sample can be refrigerated by a cooling coil right at the sample collection device to prevent degradation of any portion collected throughout the day. At the end of the 24-hour period, the sample is mixed by shaking and stored.

d. Replace urinal. (Stops and purges system).

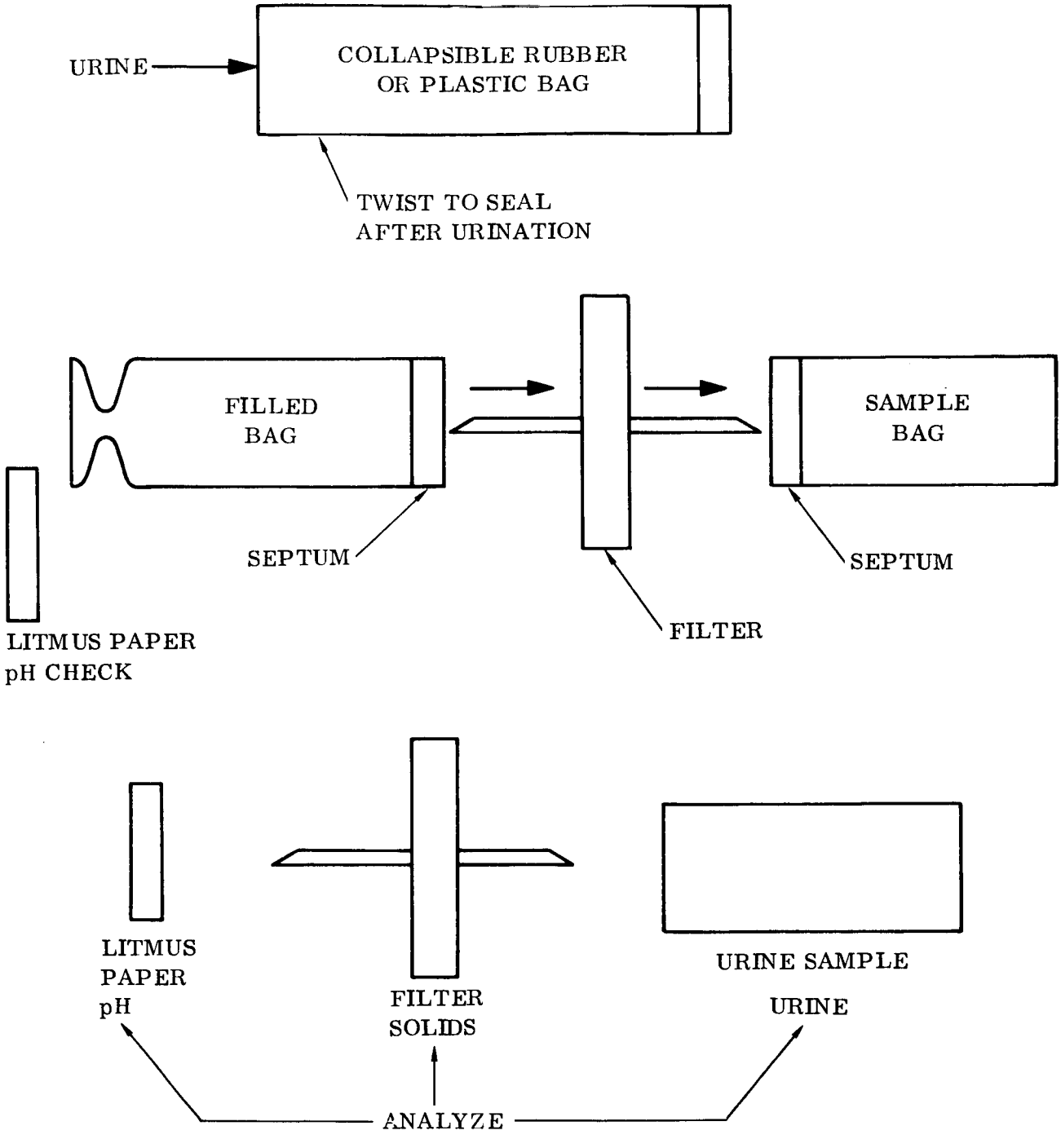

Figure 6-9. Manual Urine Sampling Technique

6.1.3 BLOOD SAMPLING

As normally performed in an environment where gravity exists, blood is collected by two methods: (1) puncture of the skin followed by drawing a few drops of blood onto a glass slide, or into a pipette or capillary tube; (2) venipuncture, employing moderate vacuums to fill a syringe or evacuated container. The first method should present no problems in the zero gravity environment of space flight. But the second method may not be useable because foaming of the blood may occur when exposed to moderate vacuums in the absence of gravity. Only a few tests are normally performed on whole blood collected by methods (1), and most of these test, such as microhemotocrit, must be done immediately. Some, but by no means all of the other tests listed in Appendix A could be made on blood collected in this way; its preservation for later analysis would present additional problems. Storage for many tests requires separation of plasma or serum from cells so that transfer of blood from a subject to a container which can be centrifuged must be effected. Method (2) may require the engineering of hardware incorporating some form of collapsed sack or plastic bag in a rigid container which could be subjected to centrifugation. Venous pressure would fill the bag with blood. An additional requirement would be a technique whereby the plasma or serum and cells could be separated and compartmented following centrifugation so that the serum or plasma would not be contaminated by the breakdown of cells during storage.

For the protection of subject and sample, aseptic handling and sampling techniques must be employed throughout. Skin, prior to puncture, must be decontaminated with a disinfectant such as 70% isopropanol, zephiran chloride, etc. Needles and containers must be clean and sterile. In those cases where whole blood or plasma must be stored, the collection containers should contain anticoagulants which are dictated by tests to be performed on specific samples; surfaces which contact the blood should be nonwettable. Siliconization or the use of nonwettable plastics would be necessary.

The collection of blood requires competence on the part of the person performing the punctures, so that neither the safety of the donor nor later experiments on samples will be jeopardized by faulty technique. Inadequate disinfection could lead to infection;

improper puncture could lead to extravasation, vein collapse and severe discomfort, with resulting drops in performance levels possible.

A further aspect of the collection regimen which must be considered is the local soreness caused by puncture would require that samples be withdrawn from a number of sites on a rotating basis. Since tactile sensitivity may be of prime importance to the astronauts involved, it may be necessary to use other sites for withdrawal of drops of blood than the fingers. The heel, ear lobe, and toes are alternatives, although the layers of cornified epithelium on the heel and big toe of the adult male make these sites inconvenient.

A good capillary supply is necessary so that the required amount of blood will flow freely from a puncture. In the case of venipuncture, it will be necessary to alternate arms and probably to use different veins on the arms. The employment of leg veins for this purpose should be considered.

It should be noted that, in order to obtain a given volume of serum or plasma, twice that volume of whole blood must be withdrawn. This is necessitated by the fact that cellular components occupy about one-half of whole blood volume.

6.1.3.1 Sampling Devices

A possible collection device (Figure 6-10) consists of a collapsed plastic bag with a hypodermic needle at each end. Each needle is protected by a sterile cap seal. In the case of plasma separation, an anticoagulant is stored at one end of the bag and is isolated from the rest of the bag by an external clip seal. An alternate device (Figure 6-11) utilizes an evacuated bag into which serum or plasma will be drawn when a valve is opened following centrifugation.

6.1.3.1.1 Plasma

The whole blood is first collected by a venipuncture with one hypodermic needle. Venous blood pressure fills the collapsed section of the bag. The needle is removed from the

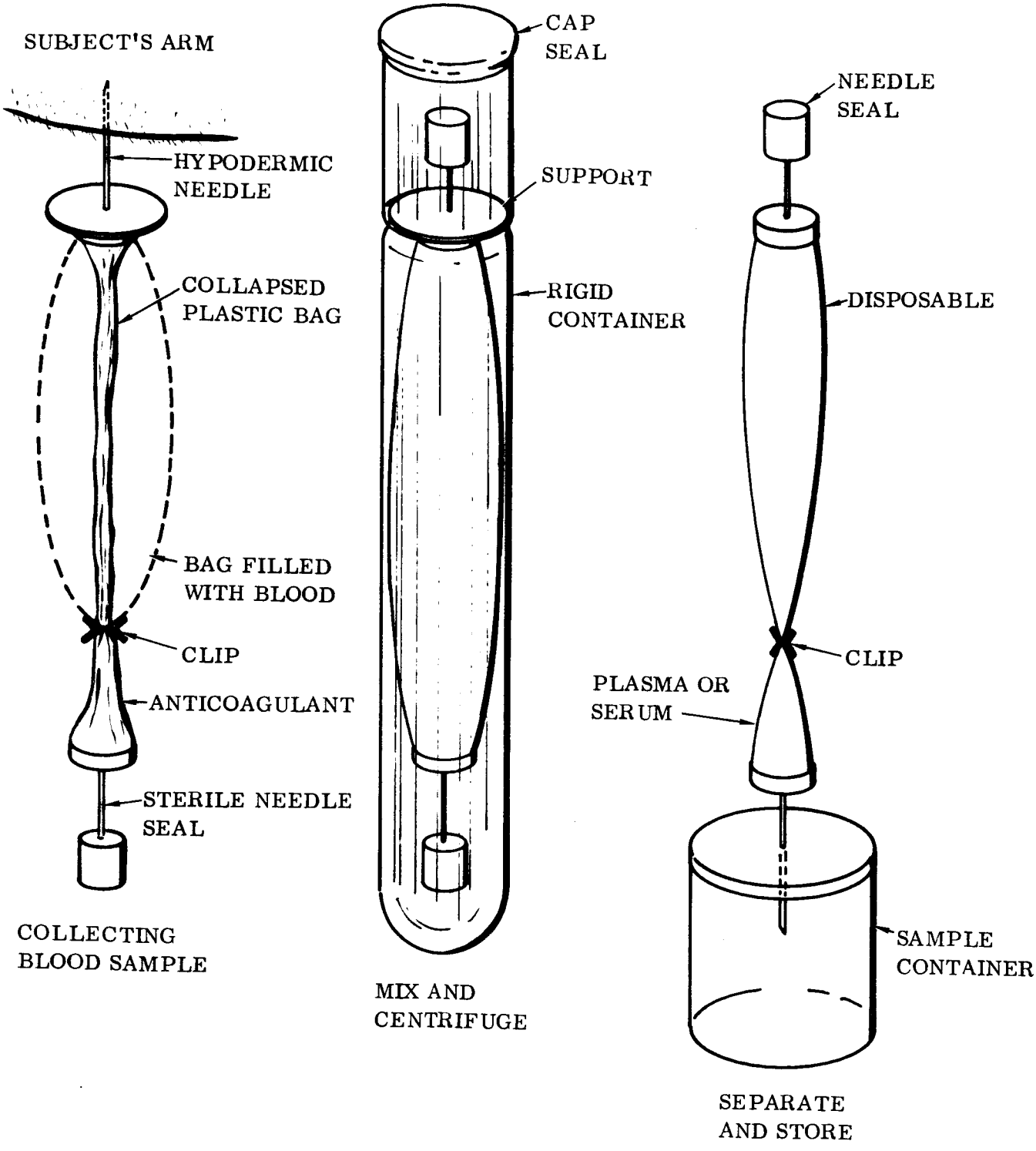

Figure 6-10. Plasma or Serum Separation Technique

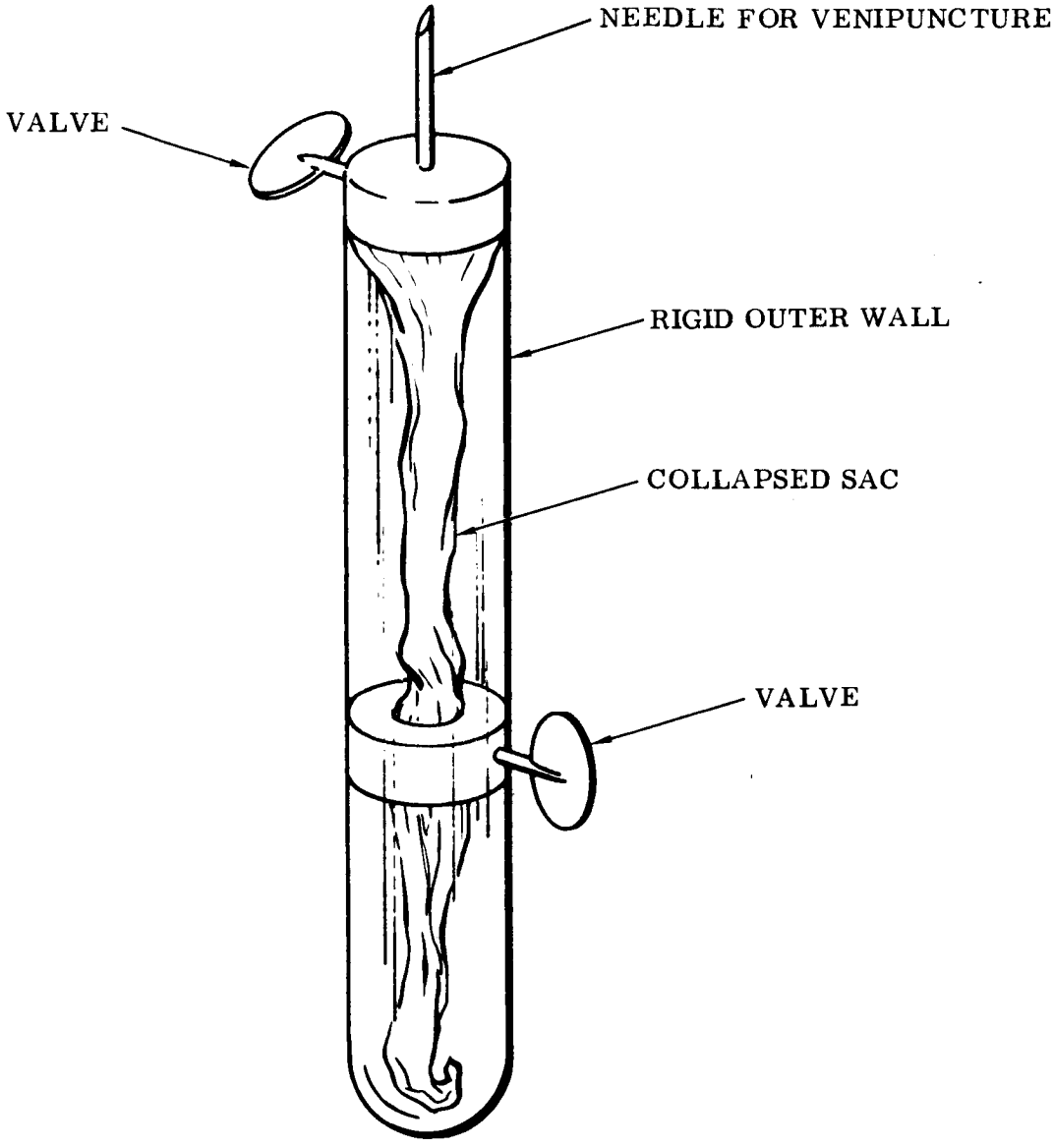

Figure 6-11. Alternate Blood Collection and Storage Apparatus

subject and is sealed by a sterile end cap. Then the clip seal is removed from the bag and the appropriate prepacked anticoagulant is mixed with the collected whole blood. The device is then inserted in a rigid support container and centrifuged. A support holds the collection device in a straight condition. After centrifuging, a separation between the plasma and the cells can be seen. The clip seal is placed at this interface thus isolating the plasma. The plasma is then removed from the collection device to a sample container for preservation and later analysis. This is accomplished by removing the hypodermic needle seal, injecting the needle through a septum and squeezing the plasma into a sample container. The collection device is then discarded (See Paragraph 6.2.4.)

6.1.3.1.2 Serum

Serum is collected in much the same way as plasma except that an anticoagulant is not used. After collecting the whole blood, the sample is left to coagulate. After clotting is completed, the sample is kneaded to breakup clots adhering to the container or the clot is loosened with a manually rotating rod (Figure 6-12) and the sample is centrifuged. Centrifuging separates the serum from the clot. The clip seal is placed at the interface and the serum is isolated. The serum is then injected into the sample container and preserved for later analysis.

It is also possible to obtain serum or plasma with a container containing only one hypodermic needle by predetermination of the end where the plasma or serum is to be collected prior to centrifuging. The illustrated technique admits blood through one needle and dispenses the plasma or serum through the other.

Capillary tubes can be used to collect blood from finger or ear lobe punctures. Hematocrits can be run in a centrifuge head which can be mounted on the centrifuge described in Paragraph 6.1.3.2.

Collection of blood for smears and clotting time determinations is discussed in Paragraph 3.1.

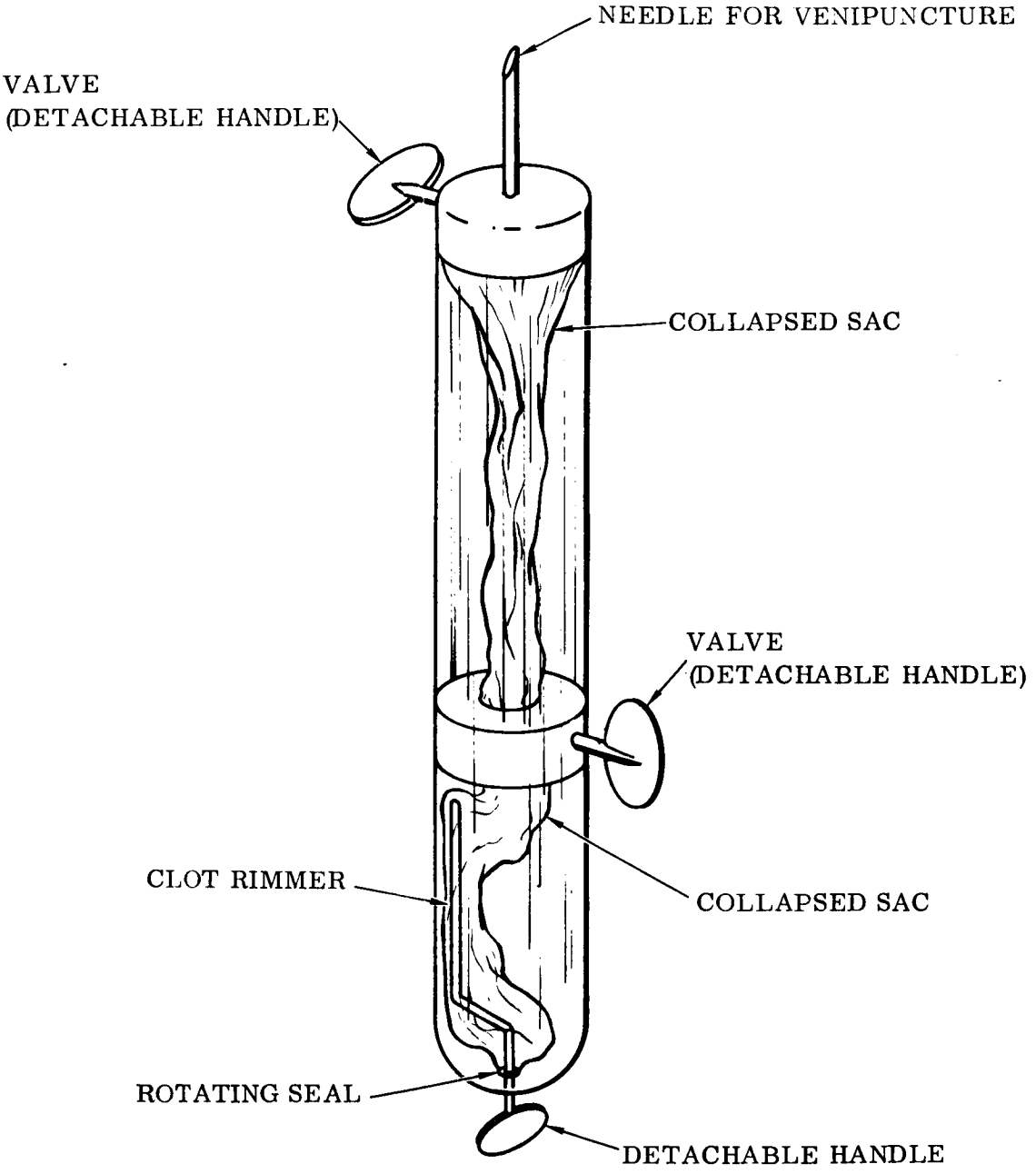

Figure 6-12. Withdrawal Container for Serum

6.1.3.2 Centrifuge

6.1.3.2.1 General Considerations

One general consideration regarding the operation of a centrifuge in an orbiting vehicle pertains to the reaction forces and torques which are introduced to the vehicle. On the one hand it appears that no design is capable of reducing the forces and torques to zero. This follows from the fact that exactly identical samples cannot be measured out, hence a residual imbalance must occur resulting in a rotating reaction force to the vehicle. Also, if it is desirable to reduce reaction torques upon the vehicle, counter-rotation which might be employed to balance out the momentum about the centrifuge spin axis cannot accomplish this perfectly since again, some error must be introduced in the measuring of the centrifuge samples.

On the other hand, the need for continuous zero reaction forces and torques to the vehicle are questionable. Preliminary information from related studies indicates that the need for absence of disturbance to the vehicle truly exists only during discrete periods of time. Instead, it is recognized that practical requirements include:

a. No operation of equipment which could result in disturbances to the vehicle during certain discrete time periods.

b. No continuous or long term disturbances to the vehicle which would be cumulative, eventually tending to saturate some element of the vehicle attitude control system.

6.1.3.2.2 Design Considerations

In the light of the requirement for not saturating any elements of the vehicle attitude control system and the characteristics of a conventional centrifuge, three types of disturbances should be considered:

a. Unbalanced rotating centrifugal force acting upon the vehicle, resulting from the mass imbalance of the samples.

b. Reaction torques imparted to the vehicle resulting from the momentum change introduced with spin-up of the samples and drive motor.

c. Precessional torques introduced to the vehicle as a consequence of the angular momentum of the centrifuge and sample as the vehicle orbits the earth.

The first of these effects, a rotating centrifugal force, does not contribute to saturation of the attitude control system since it is sinusoidal with time; hence, its average effect is zero. Similarly, the reaction torque introduced to the vehicle with spin-up of the centrifuge is cancelled by the subsequent deceleration of the centrifuge upon completion of its operation. Therefore, over one operational cycle of the centrifuge, the net effect of reaction torque upon the guidance system is zero. Precessional effects, however, are cumulative. If it is considered that the space vehicle orbits earth and maintains a fixed orientation with respect to earth resulting in an absolute angular velocity equal to its radius vector from earth, then a constant precessional torque will be generated which is equal to the cross product of the vehicular angular velocity and the momentum vector of the centrifuge.

It follows, then, that the operation of a centrifuge introduces three disturbances to a vehicle. Two of these null out with time, unbalanced reaction forces, and reaction torques. Precessional torques, however, are cumulative. The specific design of a centrifuge for a spatial application, then, must be integrated with the attitude control system. If extremely precise measurements are to be made which can be adversely affected by disturbances, the centrifuge cannot be operated at such times. Operating at other periods results in no long term effect except for precessional torques, which, because the cross product is involved, can be reduced to zero if the centrifuge spin axis is perpendicular to the orbital plane. If this cannot be arranged, two choices are available:

a. Integrate the effect of the centrifuge with the attitude control system making certain that the latter has sufficient capacity.

b. Incorporate within the centrifuge a counter-rotation element to reduce the centrifuge momentum to very near zero (except for residual errors due to inaccuracies in the samples). This then reduces the precessional torques accordingly. (See Figure 6-13.)

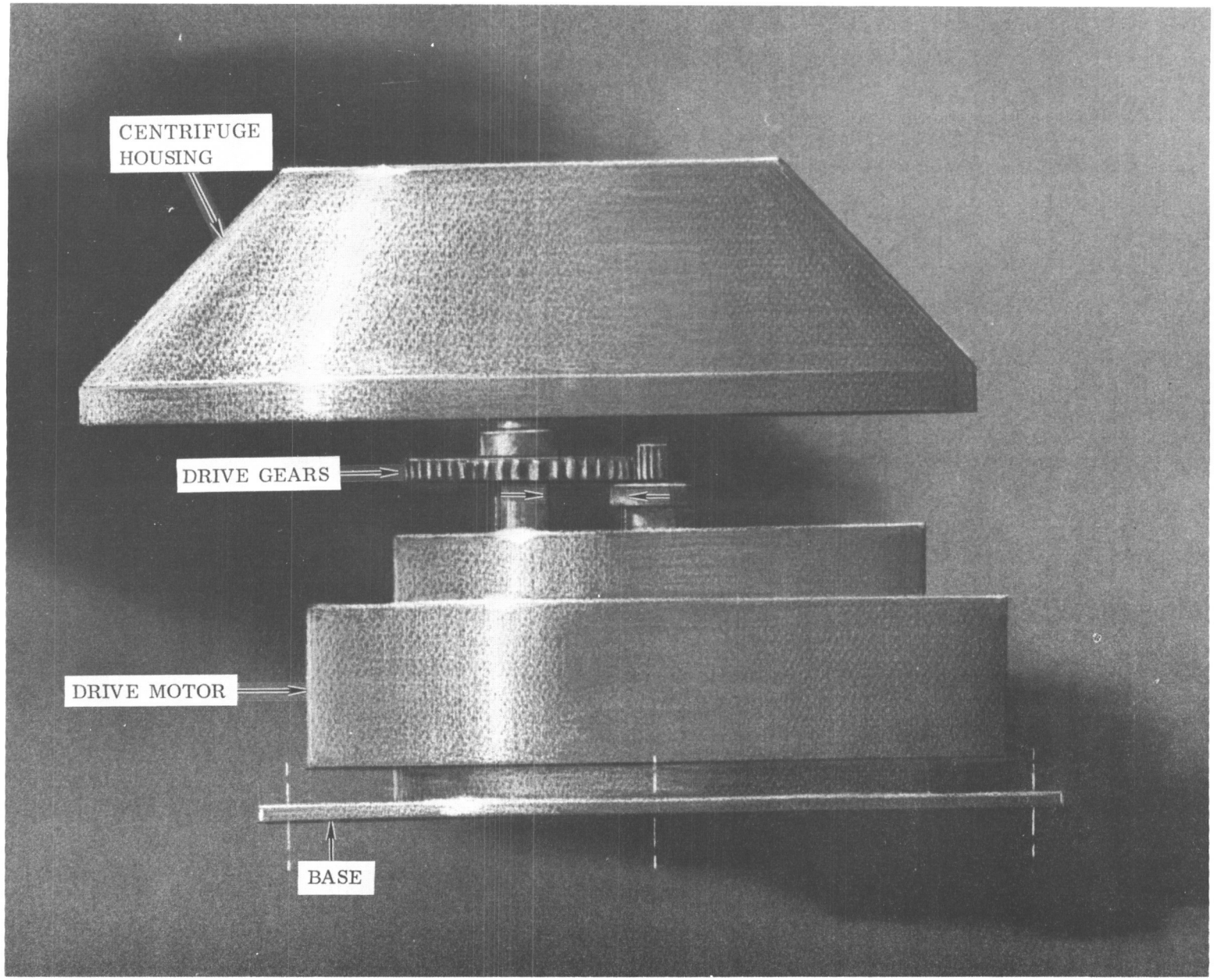

Figure 6-13. Centrifuge With Counter-Rotating Drive - Conceptual Design

6.1.3.2.3 Specific Designs

Considering the ordinary laboratory centrifuge to be satisfactory from the standpoint of the g's required, then:

$$\omega^2 r = 1000 \text{ g's}$$

With a radius of about 4 inches (1/3 ft) for the centrifuge:

$$\omega^2 = \frac{1000 \times 32.2}{1/3} = 96,600 \text{ rad}^2/\text{sec.}^2$$

and ω = 310 rad/sec. or approximately n = 3100 rpm.

Since it is desirable to accomplish this in minimum time, to reduce possible precessional effects, assume that this is accomplished in less than one minute; then the energy imparted into 4 samples of 5 ml each is:

$$E = 1/2\, Mv^2$$

$$E = 1/2 \times \frac{4 \times 5 \text{ grams}}{454 \text{ gram/lb}} \times \frac{1}{32.2 \text{ ft/sec}^2} \times \left(\frac{107 \text{ ft}}{\text{sec.}}\right)^2 = 7.8 \text{ ft lb}$$

If this acceleration takes place in one minute, virtually no power is required for acceleration beyond that required by the motor's rotor. Accordingly, power into the unit is only the no-load power required by the motor plus windage losses of the sample. This total is expected to be below 25 watts.

An artist's concept of the centrifuge is shown in Figure 6-14. It is very similar to the standard types. It is approximately 8 inches in diameter, 6 inches high and weighs 2 pounds. Power is provided by either a 400 cycle ac induction or a dc brushless type motor for high efficiency.

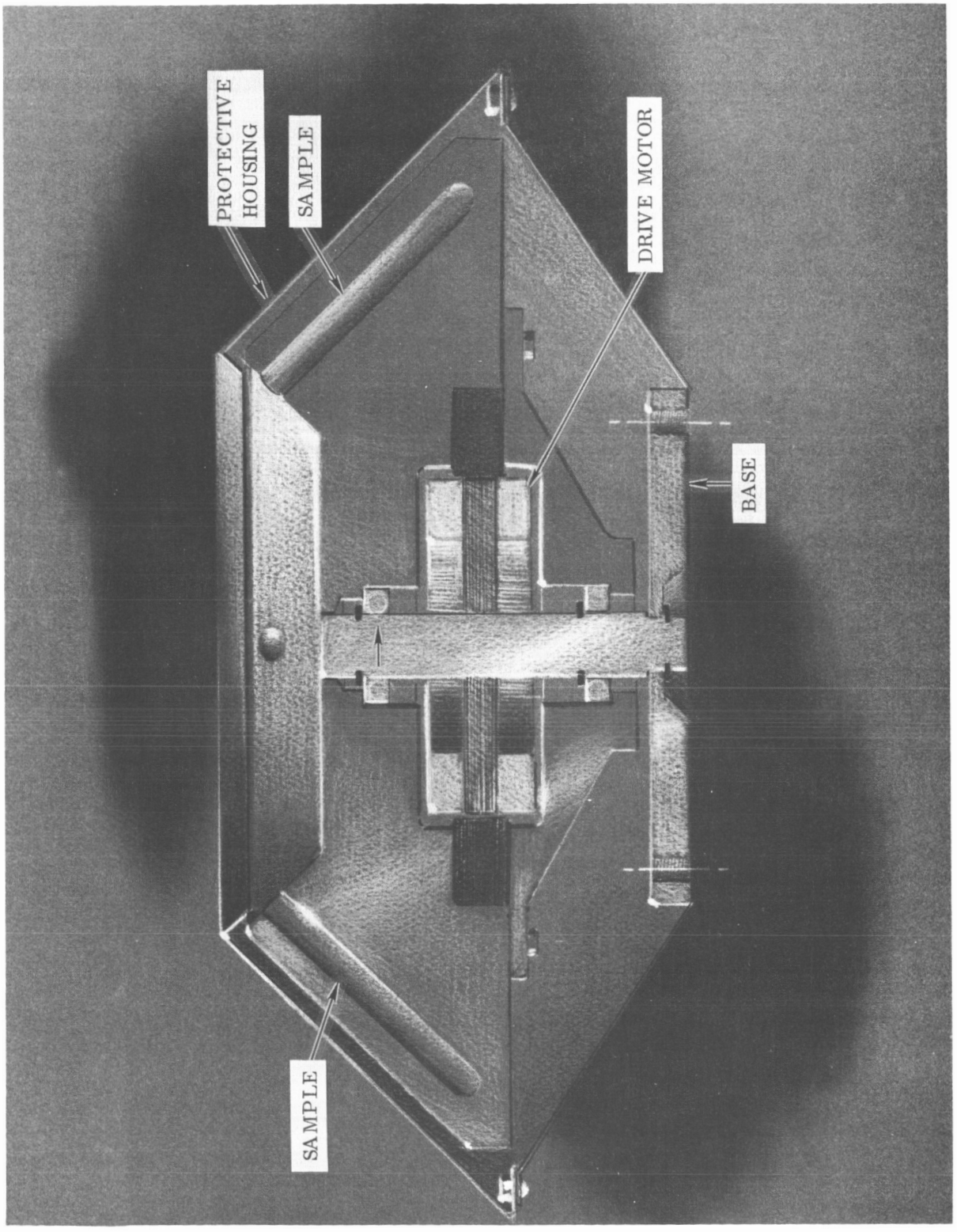

Figure 6-14. Centrifuge-Conceptual Design

After centrifuging the whole blood, the separated serum and plasma samples can be collected by capillary pipettes and stored for later analysis.

6.1.4 SWEAT SAMPLING

There are two chief approaches which can be taken to the problem of collection of sweat. First, a known volume of sweat can be collected by use of a clinical "sweat test" procedure. This involves sealing a carefully cleansed arm in a plastic bag and inducing sweating over a period of about 1 1/2 hours by placing the subject in a warm humid room or putting the other arm in warm water. In a space vehicle, a heating pad wrapped around the arm could be used. (See Figure 6-15.) This technique would yield a measure of the "instantaneous" value of electrolytes (sodium, potassium, calcium, and chloride) in a subject's sweat and could probably be modified without too much difficulty to the zero gravity environment of a spacecraft. But it would not generate information of a more valuable nature: namely, the total amount of electrolytes secreted over an extended period of time.

The second approach to collection of sweat would answer this objection. This method would collect the total volume of electrolytes secreted over a given period of time. It could be done on a continuing basis so that total electrolytes secreted during space flight could be determined. The technique would involve dressing the subject in long cotton underwear which could be removed and washed in a known volume of distilled water every 24-hours or longer. The subject's skin surface would be washed with a known volume of distilled water on a damp cloth or sponge which would also be washed at the same time. The water used would be pooled and a sample of it stored using a chemical preservative such as phenol for later analysis on the ground, or depending on electrolyte concentration, distilled and the residue saved for analysis.

This second approach would be very useful in determining the long term excretion of electrolytes during space flight. It would require an onboard washing machine whose engineering requirements have been considered. A block diagram of the system is shown in Figure 6-16.

SWEAT TEST

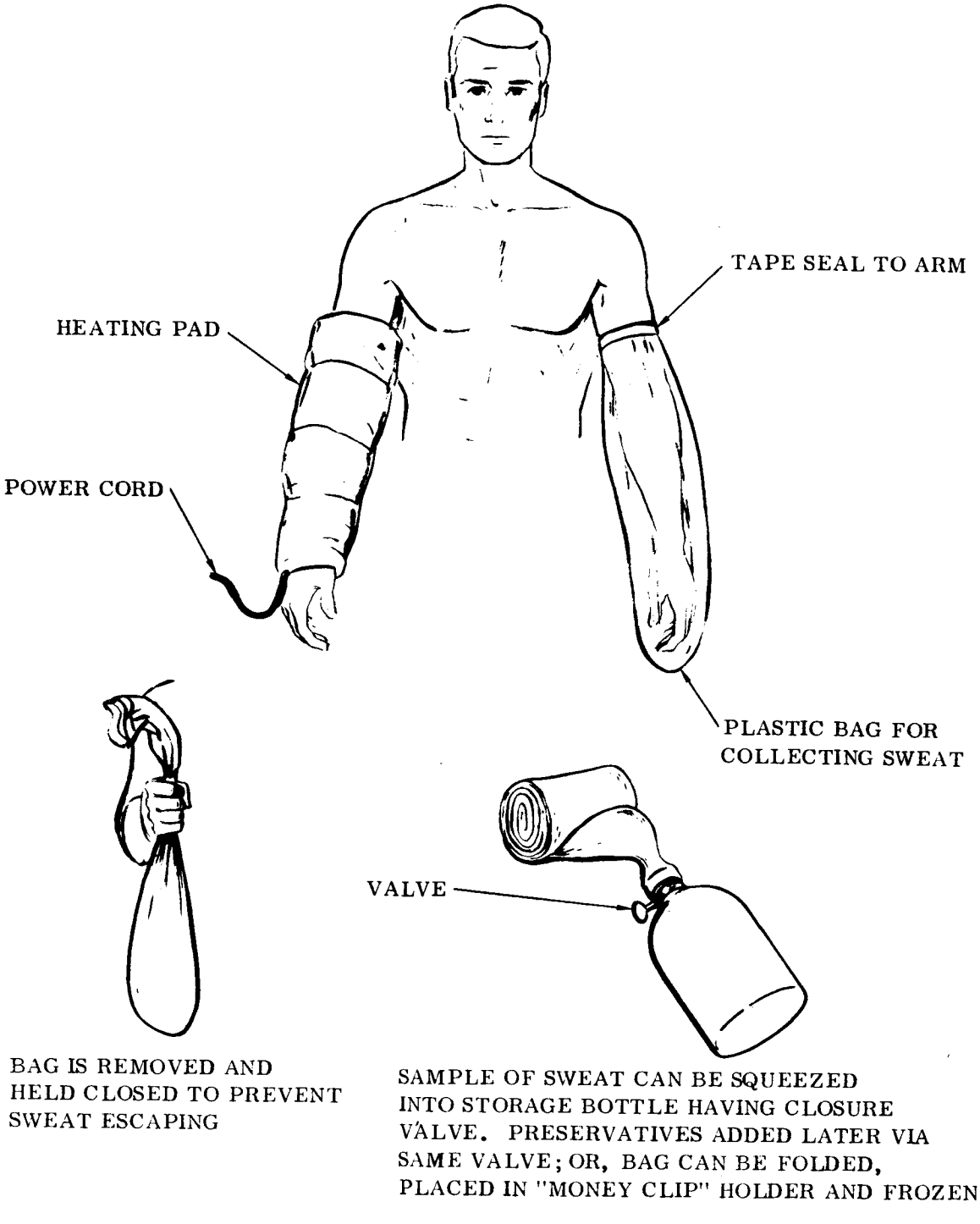

Figure 6-15. Sweat Collection System

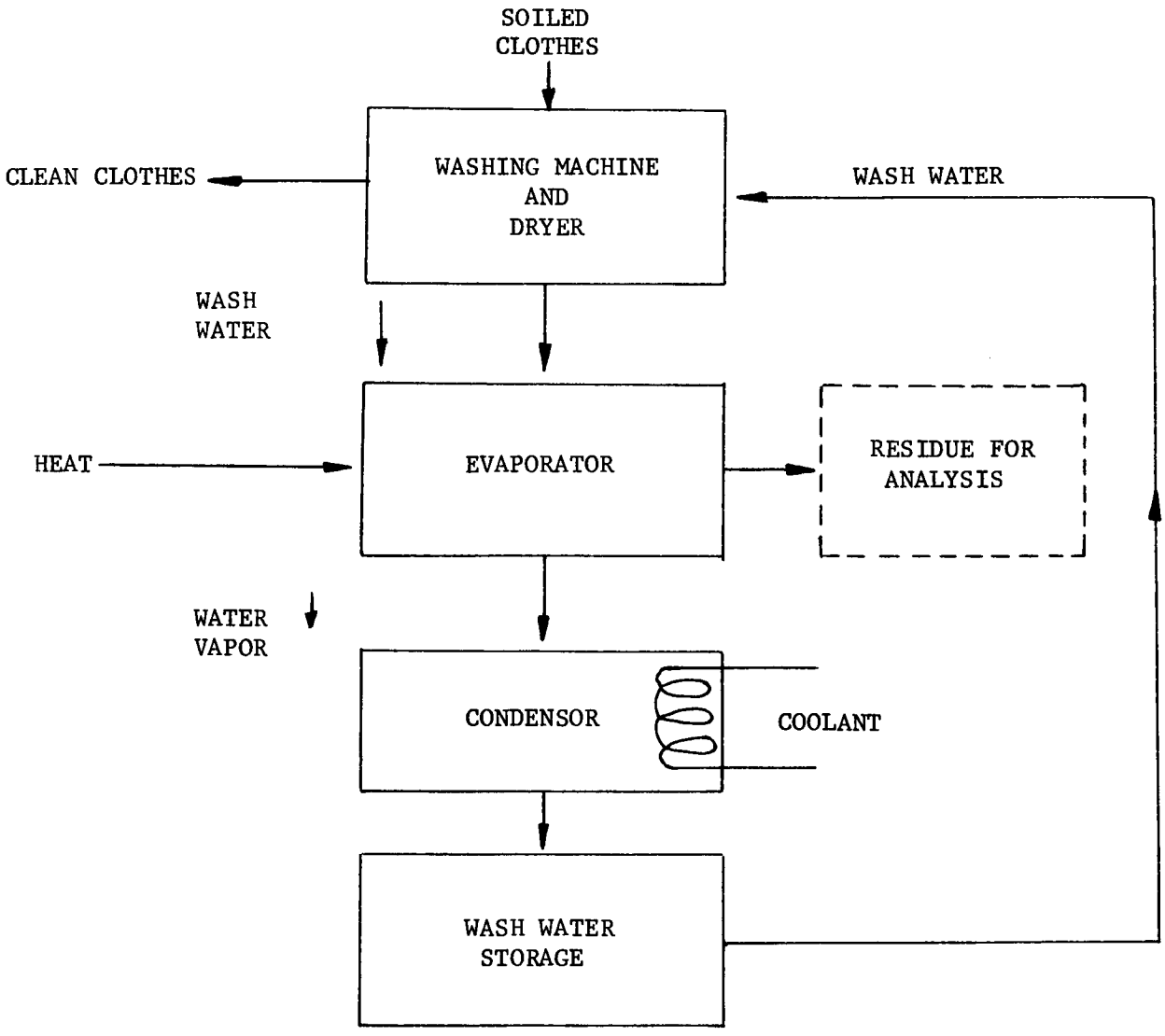

Figure 6-16. Sweat Sampling System - Block Diagram

It would also be attractive from other points of view, for example, the personal hygiene of the astronauts. The total vehicle weight would be reduced by minimizing the amount of expendable clothing required. Table 6-1 shows clothing requirements with and without washing. A preliminary washing machine design for space vehicle use is described in Paragraph 6.1.4.1.

6.1.4.1 Washing Machine

6.1.4.1.1 General Considerations

Operation of a washing machine within an orbiting space vehicle necessitates consideration of the disturbances transmitted to the vehicle, since no means appear feasible to reduce the reaction forces and torques upon the vehicle to zero. Preliminary information indicates that only during certain discrete periods of time is the space vehicle sensitive to such disturbances. Accordingly, it is considered here that washing machine operation will be restricted to those periods of time in which the spacecraft is tolerant of disturbances which accompany its operation.

Two types of disturbances must be considered:

a. Those which are rapid relative to the orbital period and tend to produce no net effect with time, such as would result with a rapid reciprocating motion.

b. Those which tend to be cumulative. This type would be produced by an operating centrifugal pump whose axis was rotated in space. This would introduce a precessional torque upon the vehicle whose magnitude equalled the cross product of the pump rotor's momentum vector and the angular velocity vector of the vehicle.

So long as operation of the washing machine is restricted to non-sensitive time periods from the standpoint of disturbances, the former type is acceptable to the space vehicle. The latter type, however, could contribute to saturation of some portion of the vehicle's attitude control system. Accordingly, operation of equipment causing the latter type of disturbance necessitates integration with the vehicle's attitude control subsystem.

Table 6-1. Clothing Weight (6 Men - 1 Year Mission)

Item	Unit Weight	No Washing		Washing	
		Items Required*	Weight (lb)	Items Required*	Weight (lb)
Shirt	0.22	104	22.88	26	5.72
Trousers	0.59	104	61.36	26	15.34
Shorts	0.15	104	15.60	52	7.80
Socks (pair)	0.04	104	4.16	52	2.08
Cap	0.04	52	2.08	26	1.04
Shoes (pair)	0.55	12	6.60	12	6.60
			112.68 lb		38.58 lb

*Including spares

6.1.4.1.2 Design Consideration

Information received from GE Home Laundry Department indicates that the work necessary to produce the scrubbing action required for ridding material of particulate dirt is 20 in.-lb/in.^{2} of fabric. For our particular case where no significant amount of dirt is involved (such as the soil in children's clothes), 2.0 in.-lb/in.^{2} should be adequate.

The amount of water required for washing corresponds to about double the amount necessary to physically saturate the garments being laundered. This amount, of course, must be repeated for rinsing operations. Tests run upon a medium weight cotton fabric indicate that 1.5 liters of water suffices for either the top or bottom of a long underwear combination outfit. This is a light weight outfit commonly used as an undergarment in a space pressure suit. Based upon 20 ft^2 (surface area of an average adult male), the energy required:

$$E = \frac{2.0 \text{ in.-lb}}{\text{in.}^2} \times 20 \text{ ft}^2 \times 144 \text{ in.}^2/\text{ft} = 480 \text{ ft-lb}$$

Assuming that the rinsing operation requires one-fourth the time required for washing, but with the same power or scrubbing action, then the total energy input required by one set of undergarments, with one rinse:

$$E_1 = 480 \text{ ft-lb} + 120 \text{ ft-lb} = 600 \text{ ft-lb}$$

and if two rinses are required,

$$E_2 = 720 \text{ ft-lb}$$

Basing the wash cycle's power requirements, then, upon 720 ft-lb total, the necessary power as a function of the total cycle time is shown by the table below:

Cycle Time	Power Input	
Minutes	Watts	Ft-lb/sec
1	16.0	12.0
2	8.0	6.0
4	4.0	3.0
8	2.0	1.5

Trade-off studies are necessary to optimize the design from the standpoint of power, weight, volume, water consumption, etc. At this time, however. it is reasible to consider a five minute cycle consisting of a four minute wash with one 1-minute rinse. The input power delivered to the water - clothes combination is four watts. Since these calculations are based upon 20 ft^2 of undergarments, this is adequate for the simultaneous washing of top and bottom. Hence, the water required is six liters for the total wash cycle with a 50% void volume in the washer during each cycle.

6.1.4.1.3 Overall Design Considerations

A number of questions must necessarily be answered to develop a space washing machine which will approach the optimum for this application. Some include:

a. Recognizing the desirability for conserving water and power, should tops and bottoms be washed simultaneously? That is, would it be desirable to wash bottoms, say, twice as often as tops and if so, then probably the machine should be sized accordingly.

b. Are showering facilities to be provided? If so, it seems reasonable to attempt to integrate the water usage, reusing the shower water for the first wash.

c. The GE Home Laundry Department has advised that special detergents could be formulated which would be advantageous for this application, which requires only a single rinse. How would these detergents lend themselves to subsequent de-ionization of the water and also recovery of the desired substances for the biological analyses?

d. Should not the washing machine be integrated with a dryer? If so, a trade-off study would be necessary to optimize the water dumped to vacuum (making the drying very simple, with little power), versus recovering all of the water in the clothes at the cost of power.

6.1.4.1.4 Design Approaches

a. Scrubbing Action - The required scrubbing action in zero "g" can be readily provided by three different techniques:

1. An array of jets at each end of a cylindrical vessel or "tub" arranged to inject the detergent solution alternately from one end to the other, thus pushing the wash load before it and impinging it upon the other end of the tub. This technique would employ one or more scavenge pumps to circulate the fluid to the jets, with control accomplished fluidically.

2. A "Bellofram" sealed piston with a porous mesh head, arranged to reciprocate within a cylinder would "pound" the garments upon the closed end, much like the technique of flailing clothes upon rocks in a river. Displaced liquid would flow through the piston head during forward stroke and back during the return stroke.

3. Continuous rotation of a drum in conjunction with a water jet can provide the necessary scrubbing, with the jet element arranged to dislodge the clothes and direct them to impact upon another portion of the drum, much like today's household tumbler washer, but using a water jet rather than gravity to dislodge the clothes and water from the rotating drum. This technique has additional advantages in that it readily lends itself to pumping out the water after the wash and rinse cycles, lends itself to a drying cycle, and perhaps even more important, uses less water.

b. Water Ejection - Water is ejected from the garments in the first two scrubbing technique configuration by squeezing the clothes between a moveable piston and the end of the cylinder. In the tumbler, water ejection would be accomplished simply by stopping the water jet and spinning the drum, centrifuging the water out.

6.1.4.1.5 Conclusions and Recommendations

At this time, the best washer type would be the tumbler unit. Its advantages include less water consumption, the ease of addition of a drying cycle and the simple means of water ejection inherent with its design. Accordingly, a layout illustrating the conceptual design for this configuration is shown in Figure 6-17.

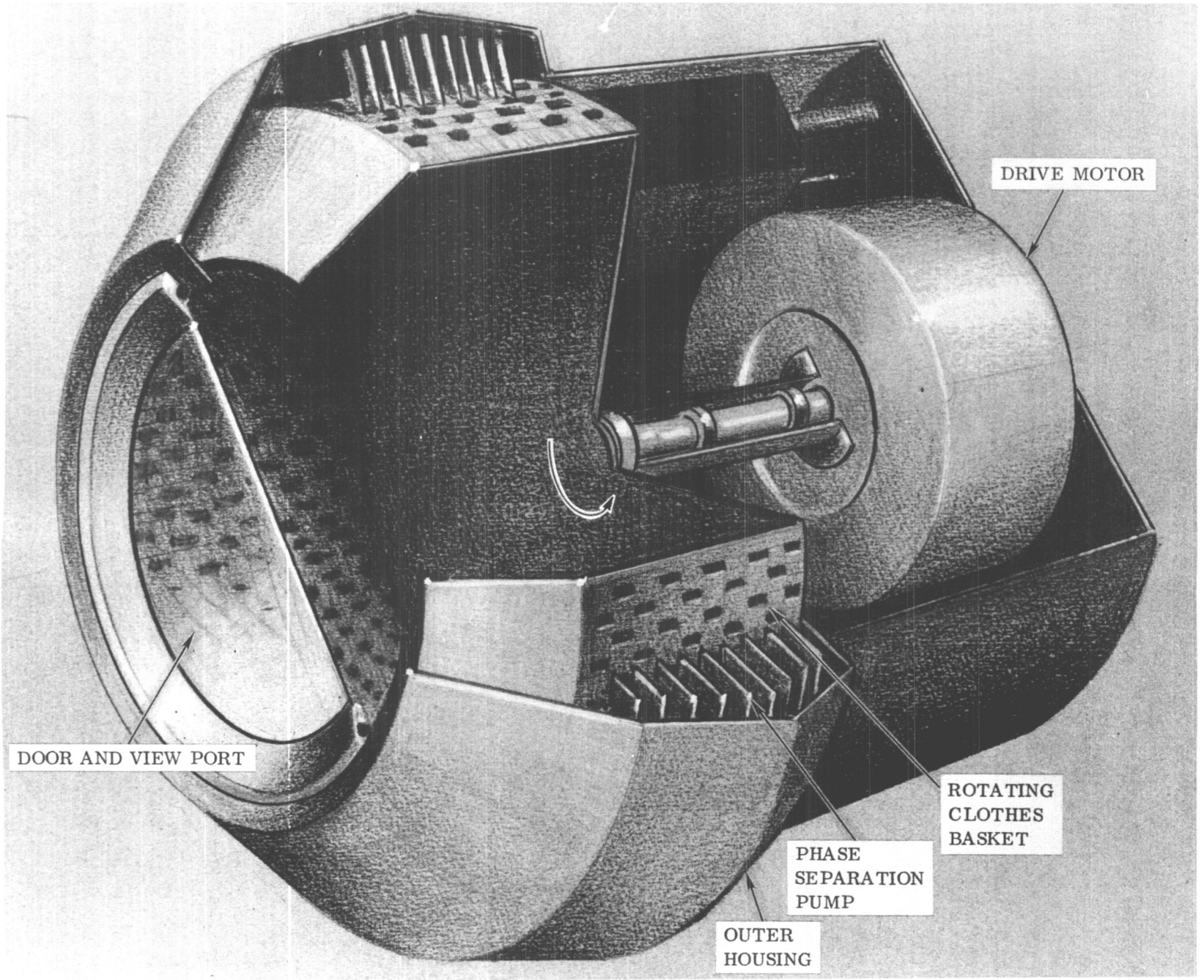

Figure 6-17. Washing Machine - Conceptual Design.

It is apparent that consideration of washing undergarments, shoes, caps, etc., raises many questions with regard to system optimization. That is, considerable savings can be effected in water and power if the overall requirements for hygiene can be treated, rather than just a single task. Accordingly, it is recommended that further study be effected for this overall task, whose goals would be:

a. Optimize the equipment necessary for personal hygiene, including showering, and laundering equipment, to effect minimum consumption of water and power.

b. Determine how drying is to be effected, trading off power and water loss (the latter if residual water is vented to space), factoring in the effects upon the atmosphere, to include even the beneficial effects of negative ions in the air (whose concentration could be increased by judicious design and operation of equipment).

The tumbler washing machine drawing shown in Figure 6-17 is sized to wash one set of long underwear (cotton), in a zero gravity environment and is estimated to incorporate the following characteristics:

Diameter	- 12 inches
Length	- 10 inches
Weight	- 14 pounds
Power Required	- Less than 20 watts
Water Required	- Less than 6 lb/cycle

For the purposes of development it is particularly advantageous to utilize the concept shown, since it can readily be shown that operation in one "g" will be like operation in zero "g". Accordingly, development time and costs are expected to be relatively low.

6.1.5 BACTERIOLOGICAL SAMPLING

One method of bacteriological sampling is accomplished by rubbing a sterile swab on the area to be sampled. The contaminated swabs are then placed in a broth culture medium and inoculated. Aliquots of the broth culture are transferred to sterile broth or to agar culture media and incubated. The incubator is also the storage and transportation module for the bacteriological samples. (Refrigeration of bacterial samples is descussed in Paragraph 4.4.2.)

6.1.5.1 Swab Holder

In order to avoid contamination of bacterial samples, swabs are individually mounted in tubular holders and prepackaged in sterile envelopes. The envelope seal is broken by pulling the sides apart below the seal. The area surrounding the seal is not handled, minimizing the possibility of contaminating the sterile swab when it is withdrawn.

The holder, an instrument 3/8-inch in diameter and 4-inches in length, allows samples to be taken without the necessity of handling the swab itself. After the sample has been obtained the swab can be released by means of a stud at the end of the holder. Holders are discarded and/or re-used only after sterilization.

6.1.5.2 Sample Containers

The container into which the swab is released is conical in shape, two inches in diameter and two inches high. Both the container and its sealing cap are moded in one piece to facilitate handling. (See Figure 6-18.)

The cap attached to the base of the container by a flexible stalk, snaps onto the rim to form an airtight seal. Unsealing and re-sealing can be accomplished without handling or contaminating the inside of either the cap or container. A one inch diameter permselective membrane is mounted to the center of the cap to permit oxygen permeation of the sealed container without the possibility of broth leakage or contamination of the external surface.

The broth culture medium is retained in a conically shaped vessel with the broth collected at the apex. (See Figure 6-19.) This is the minimum energy configuration for any liquid which wets the walls in a zero gravity environment. Extensive zero "g" drop tower tests conducted by NASA-Lewis Research Center have confirmed the theory that liquids will position themselves at the cone apex. Also, a flight experiment in the Mercury MA-7 spacecraft showed that the liquid position in a similar configuration was unaffected by the vehicle maneuvers. Consequently, the surface tension of the broth will assure the proper positioning in the vessel.

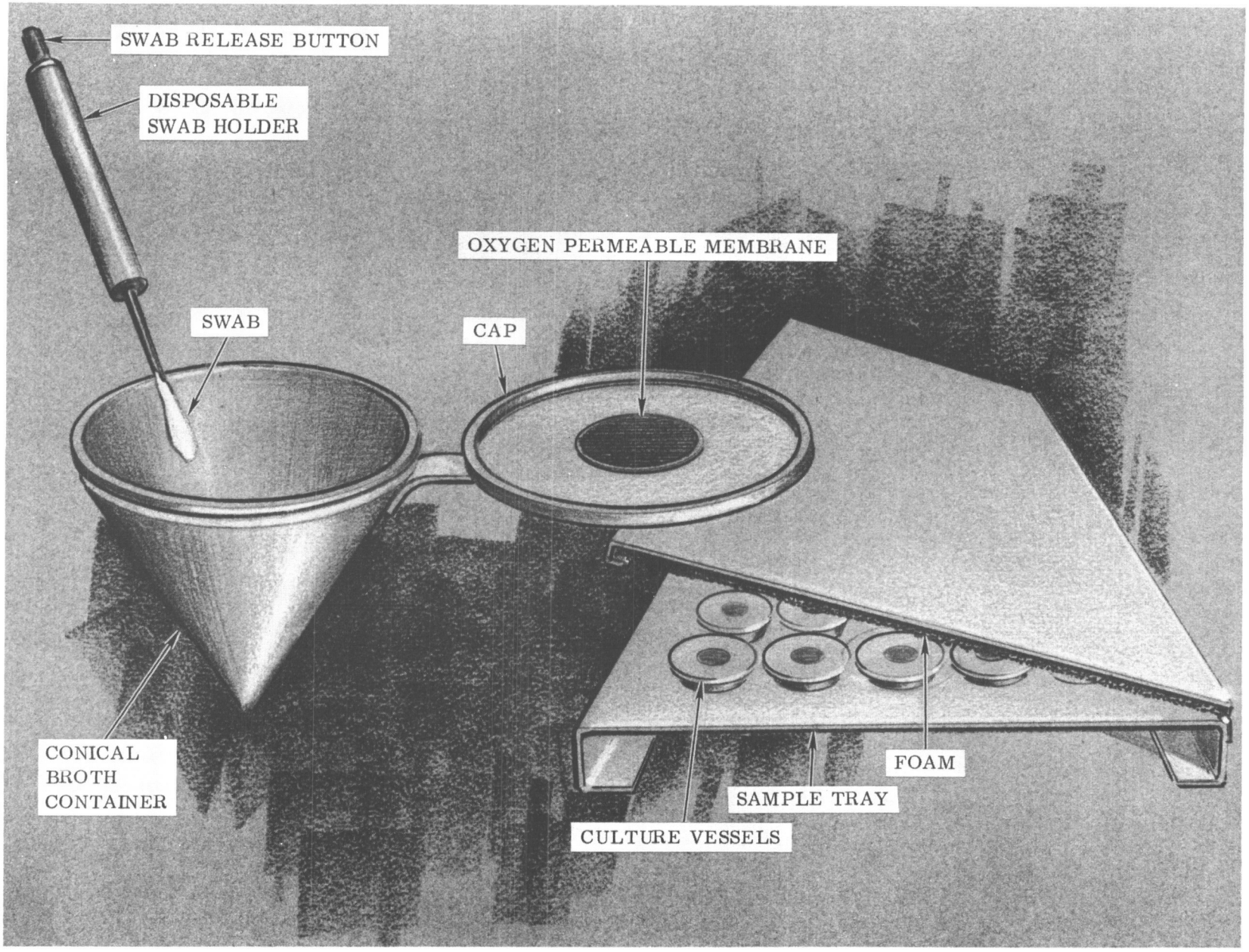

Figure 6-18. Culture Vessel for Bacteriological Samples

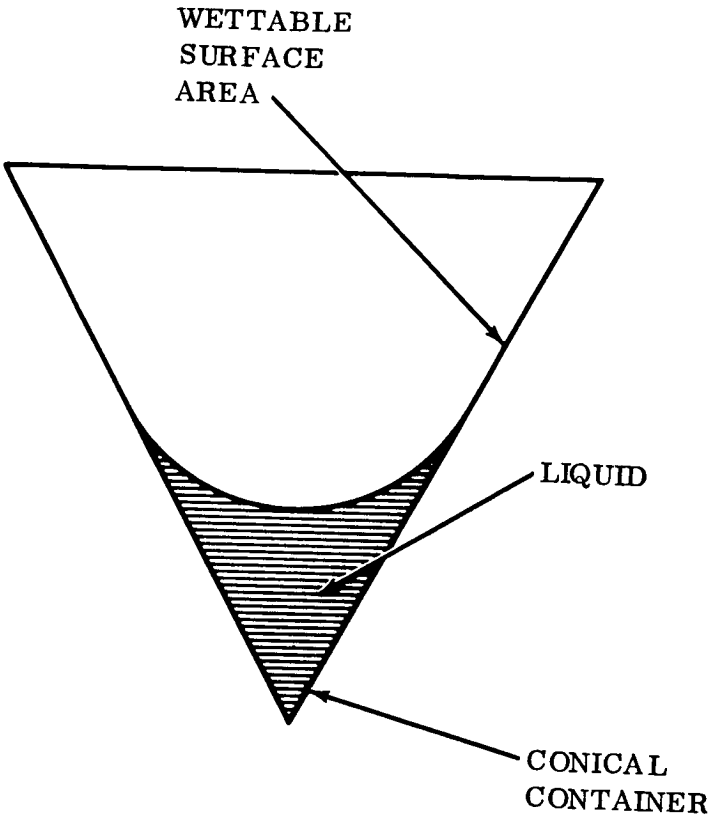

Figure 6-19. Liquid Configuration in Zero Gravity Environment

Since the culture medium vessel will probably be constructed of plastic, it may be necessary to fluorine etch the cone apex area to assure good wetting of the surface by the broth.

6.1.5.3 Retaining Tray

A tray containing conical cavities retains the sealed container during transport and storage. (See Figure 6-18.) Low density polyurethane foam padding bonded to the inside surface of a hinged lid limits container movement and maintains the cap seal.

Each tray measures 16 by 20 inches and has a capacity of 60 containers. Six trays stack within the transport module to provide a transport capacity of 360 containers per storage module. The module is transported to earth for analyses.

6.1.5.4 Incubator

The incubator and storage module is designed to store both petri dishes with agar culture media and the conical vessels with broth media.

The petri dishes can be nearly any shape, but are assumed to be a circular shape (2-inch diameter by 0.5-inch thick) to assure proper sealing and will weigh approximately 0.05-pound each. The conical vessels are 2-inches in diameter and 2-inches high and will weigh approximately 0.05-pounds each. The incubator will be an insulated box similar to those used for storing refrigerated and frozen biological samples. (See Paragraph 5.3.2.) Heat will be supplies to the box from waste heat sources within the vehicle. If a Freon vapor cycle freezing unit is utilized for sample preservation, the waste heat from the condenser would be a good heat source. The insulated box weighs approximately 1-pound for every 2000 cm^3 (0.073 ft^3) of storage volume and the total box volume is approximately twice the required storage volume. Each bacteriological sample in a petri dish requires approximately 35 cm^3 (2 $in.^3$) storage volume and each bacteriological sample in a conical vessel requires approximately 120 cm^3 (8 $in.^3$); therefore, total system weight and volume are determined in Figures 6-20 and 6-21. No electrical power is required if waste heat is utilized.

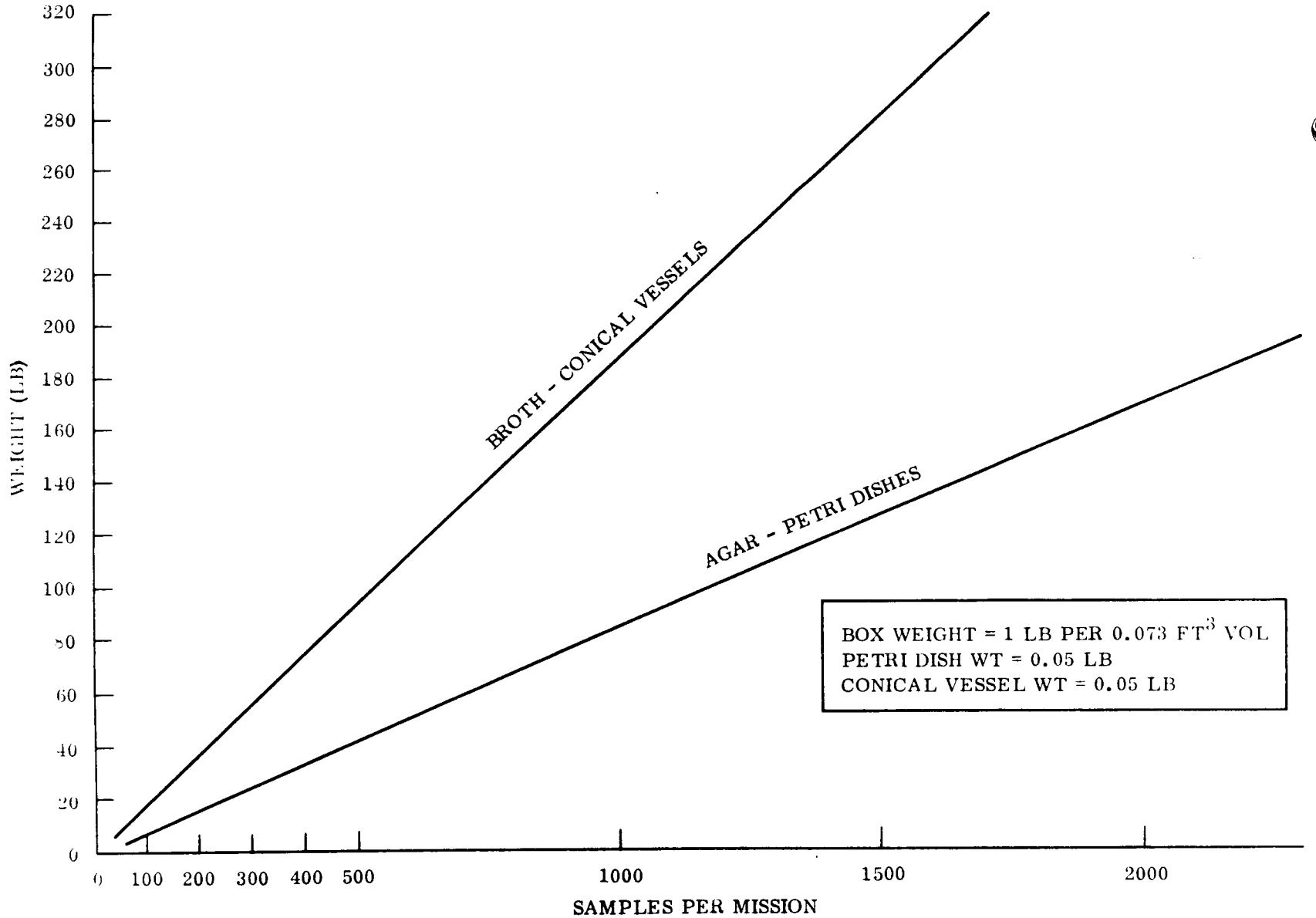

Figure 6-20. Incubator Weight for Bacteriological Samples

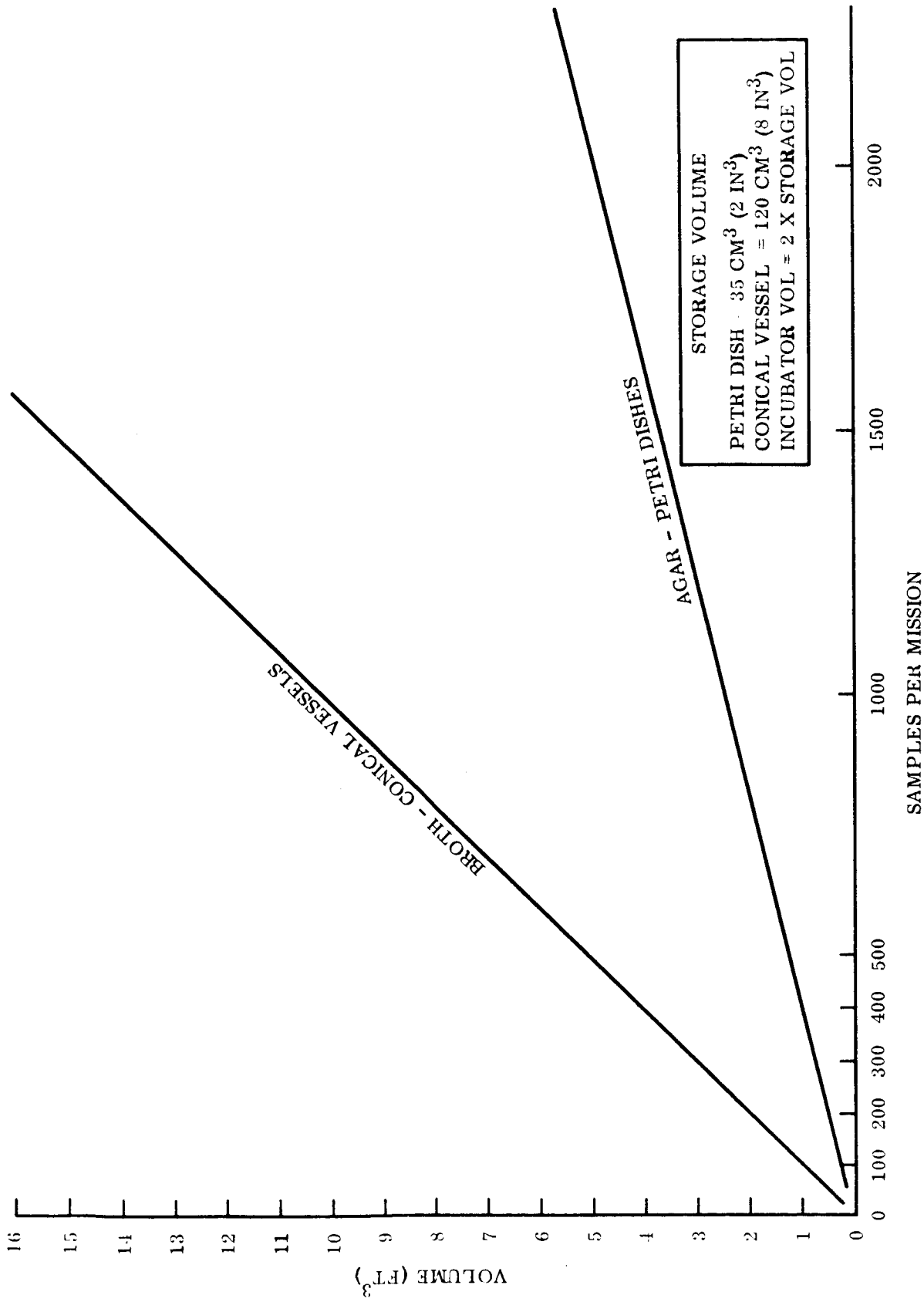

Figure 6-21. Incubator Volume for Bacteriological Samples

6.2 HANDLING

The biological samples will be handled in ten main stages from collection to final analysis as illustrated in Figure 6-22. The collection and preservation stages were discussed in the previous section; the remaining stages will be duscussed in this section.

Refrigerated and frozen samples may require temperature control during transportation. Dry ice packaging may suffice in commercial airlines where coolant and/or electrical power is not available. After sample recovery, the samples are flown to the NASA Flight Experimenter Station where they are separated for shipment to the individual experimenters for analysis. The quickest and safest means possible shall be used to transport the samples. The storage and shipping modules should be returned to NASA for refurbishment and reuse.

6.2.1 STORAGE

6.2.1.1 Sample Packaging

The main problems with sample packaging are:

a. The sample must be maintained in a sealed container so it cannot be contaminated by the environment or the container and so it cannot contaminate the environment.

b. The sample must be preserved, i.e., all constituents should undergo minimal change in value.

c. Sample foaming must be minimized. Zero gravity tests conducted at NASA-Lewis Research Center have shown that liquid will readily foam if injected into an air filled container. Thus, special sample container designs must be utilized.

These special sample containers may be of either a flexible or rigid construction:

1. Flexible Containers - A collapsed bag which expands as the sample is added is ideal for minimizing foaming in the zero gravity environment. The bag can be either flattened or rolled-up for initial storage.

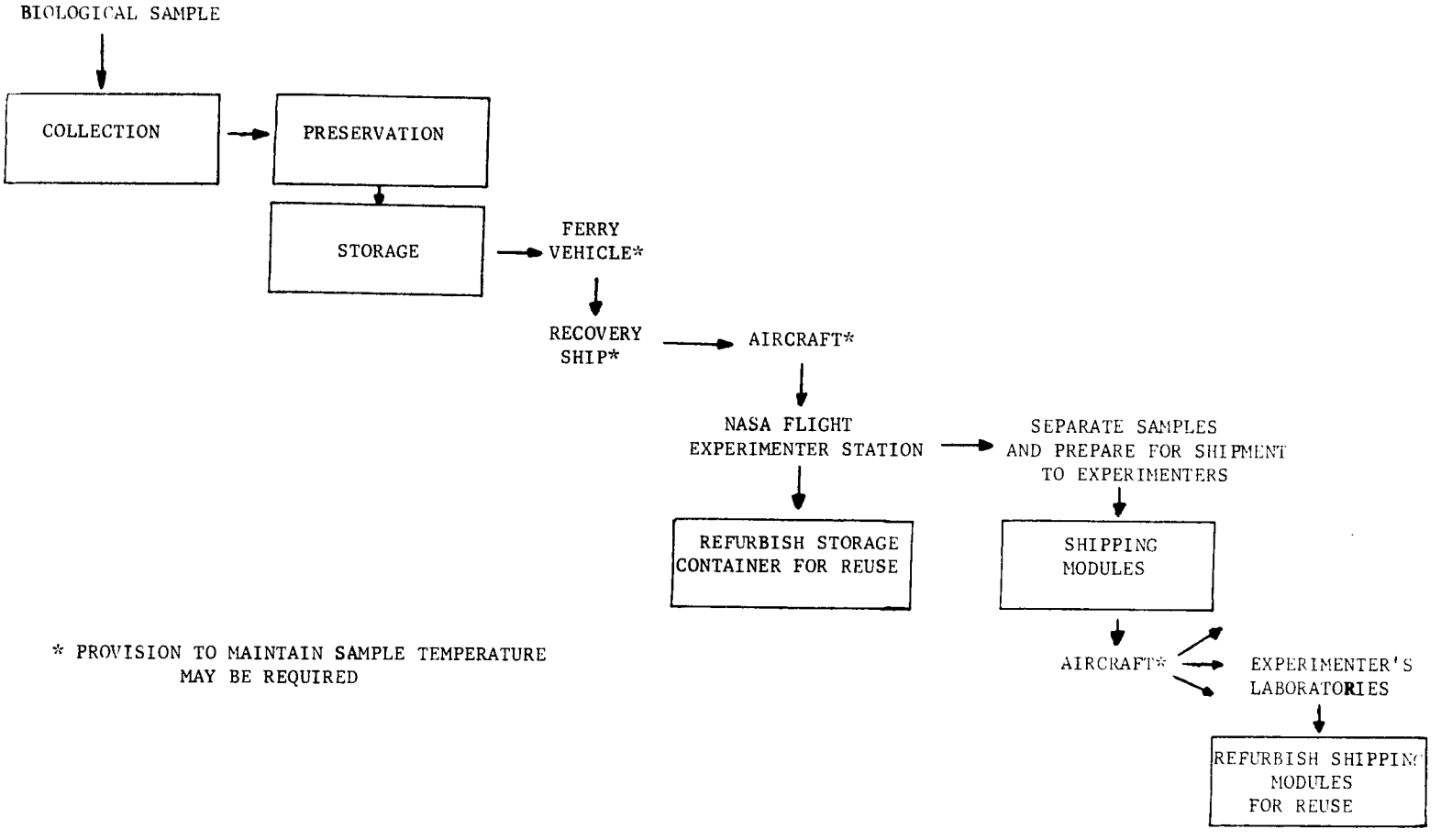

Figure 6-22. Biological Sample Handling Requirements

2. Rigid Containers - Rigid containers must be evacuated or filled with a highly wettable foam or wicking material to prevent sample foaming in the zero gravity environment. The container shape may be varied to nearly any configuration.

   Either type of sample container may be designed to integrate with any of the preservation techniques. However, for chemical, vacuum distillation and lyophilization preservation techniques, the flexible container is recommended to minimize initial storage volume. For refrigeration, freezing and ion exchange preservation, a rigid container is recommended since storage volume will be initially available in the sample storage module. Also, the rigid container is more amenable to rapid heat transfer from a cold plate.

3. Sealing - The flexible sample container may be sealed by heat sealing, adhesive patches, adhesive sealants (e.g., GE-RTV), sonic welding, mechanical clips, self-sealing septums, etc. The rigid container may be sealed by screw cap, snap caps, adhesive patches, adhesive sealants (e.g., GE-RTV), self-sealing septums, etc. Any of the above sealing techniques should be acceptable when properly applied. Note that when adhesives are used, the outgassing products must be nontoxic and nonirritating to the crew and must not interfere with the analyses.

d. Pressure Changes - One of the problems which will have to be investigated in preparation for AAP flights will be the effect of pressure changes upon containers used for storage of biological fluids. For example, if a flexible container is used to store sweat samples obtained from induced sweating, vacuum tight seals would be required for the chamber in which this container is stored if the cabin is depressurized for EVA or else the container would swell with entrapped gas and burst. An alternative would be the use of rigid containers, tightly sealed, which could withstand the ΔP generated by depressurization.

   Freezers and refrigerators would have to be designed with vacuum tight seals or would require built-in design factors to allow for escape of atmosphere and adequate cooling of specimens in the absence of atmosphere. The presented freezer and refrigerator designs rely on conductive heat transfer so that a convective atmosphere is not required for cooling. Incubators would require vacuum-tight seals because the growth of micro-organisms contained depend on the present of a gaseous atmosphere. Special valves may have to be built into these chambers to allow reduction of increase in pressure of contained atmosphere to cabin transient pressure when this changes from 15 psi to 5 psi or back from 5 psi to 15 psi when the spacecraft leaves earth's atmosphere and is returned to it. This may be necessary to open the doors safely.

An additional problem is generated by the difference in pressure between the pressurized cabin of the spacecraft (5 psi) and the atmosphere of groundbased laboratories (15 psi) where reagents are bottled for flight, containers are fabricated and prepared for flight, and returned samples are opened for analysis. Opening of reagent bottles against a pressure differential may result in spilling of contents even if use is made of sealed rubber septa to be entered by needle-tipped syringes. Containers used for storage of samples at 5 psi will have to be designed so that they can be opened at 15 psi in ground-based laboratories without loss of contents. Conventional closures may require modification in order to open them in the face of 10 psi pressure differential. A series of laboratory tests are required to define the problem and to prove design approaches.

6.2.2 STORAGE MODULE

The samples preserved by chemical, vacuum distillation, ion exchange, and lyophilization techniques require no special storage module. These samples can be stored in emptied food bins and will be transported to the earth in the same container after resupply.

Rubber bladders will be inflated in each storage container to assure retention of sample integrity during re-entry and recovery environments.

The samples preserved by refrigeration and freezing will require properly engineered storage modules to assure rapid cooling and minimal heat leakage, which, in turn, will hold power, weight, and volume penalties to a minimum.

A possible specimen tray arrangement and sample container module design is shown in Figures 6-23 and 6-24.

6.2.2.1 Specimen Trays

The specimen tray consists of a semirigid draw formed plastic shell covered with a flat transparent sheet of similar material sealing the cavities. The base sheet contains 36-cavities, each having a volume of 32 cc, including the antifoaming material, and may be color coded to facilitate handling and sorting. Each tray of cavities measures 18 by 23-inches and is approximately one-half inch in depth.

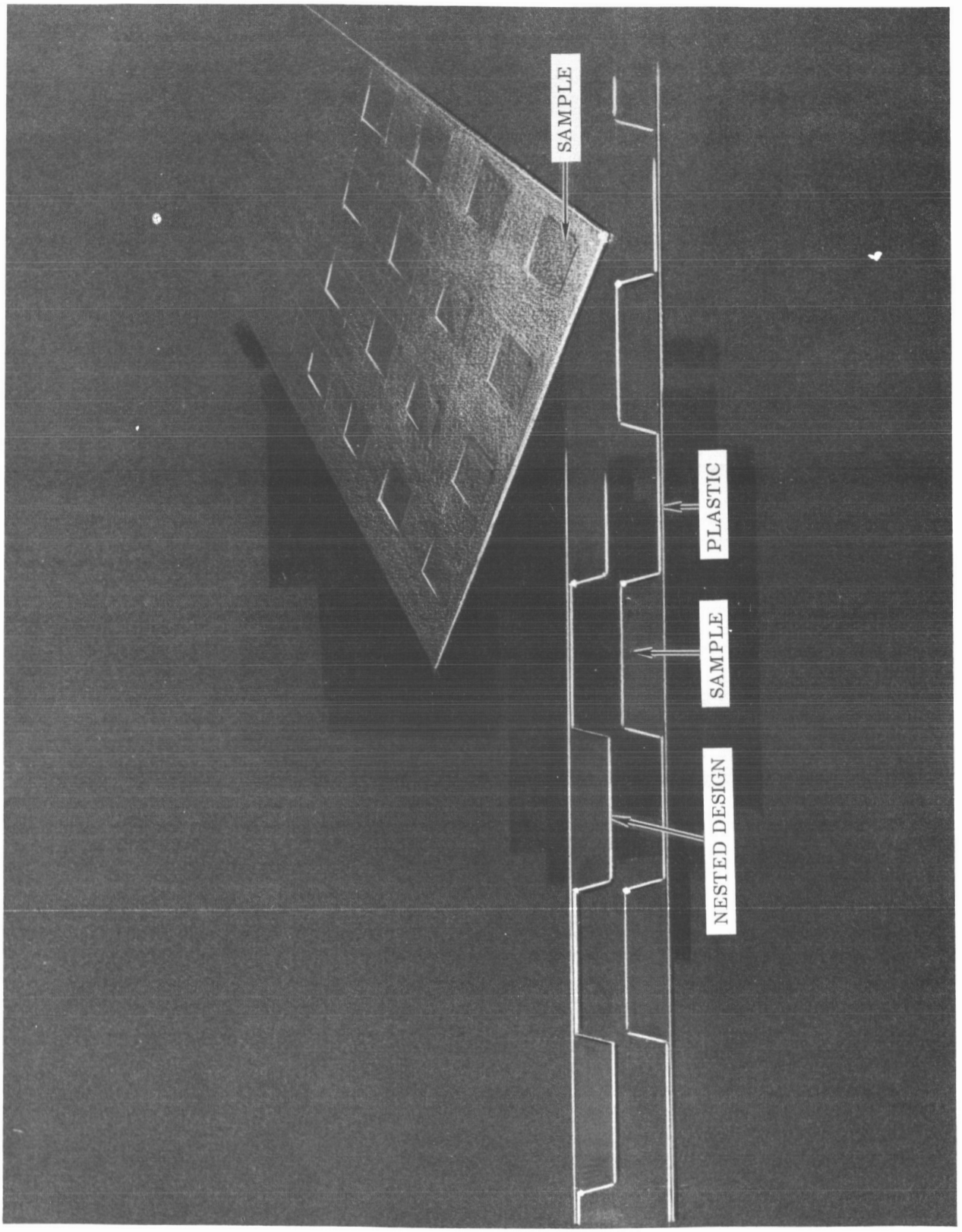

Figure 6-23. Sample Tray-Design Concept

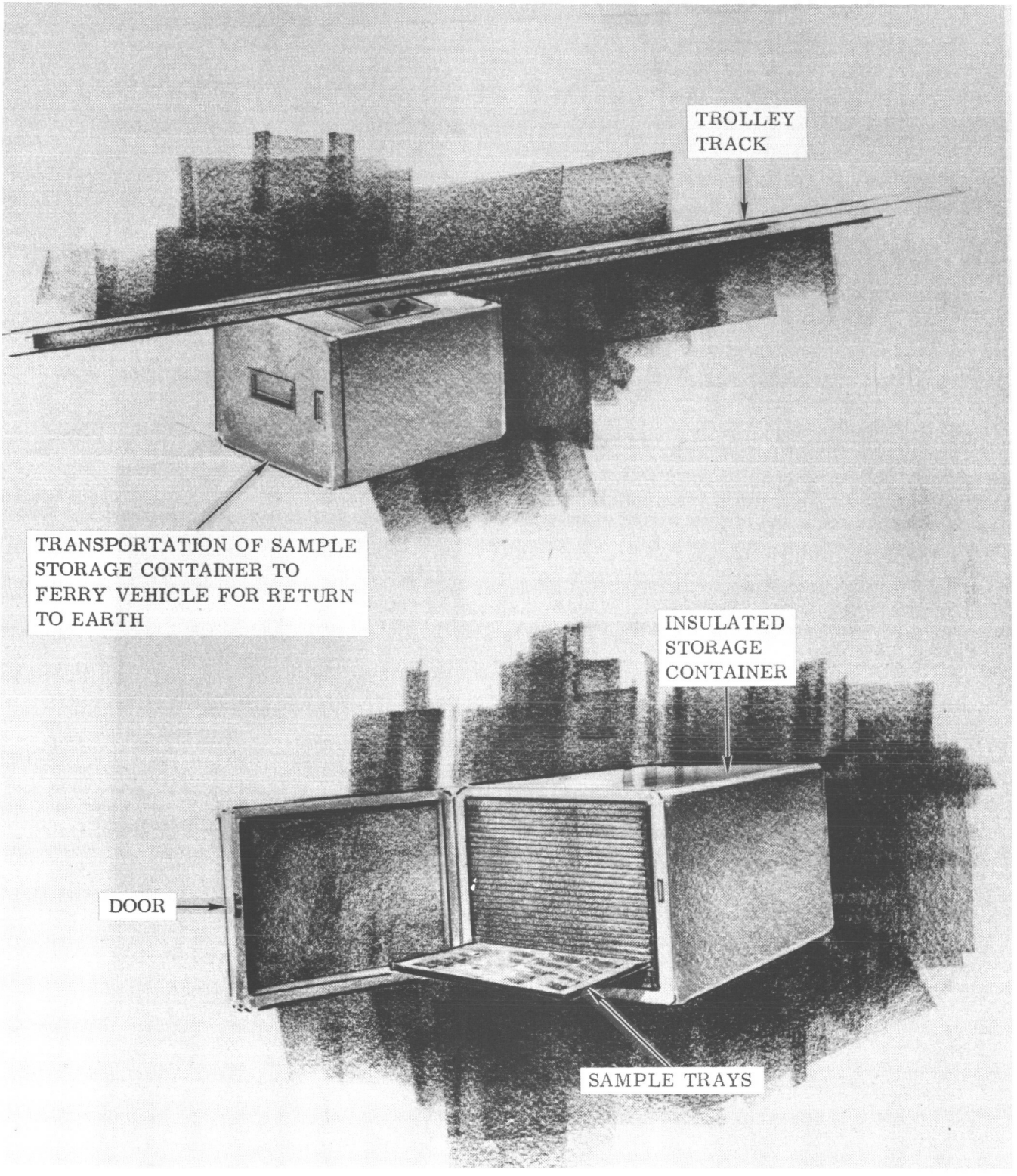

Figure 6-24. Sample Storage Module-Design Concept

Liquid samples are injected into presealed cavities. The cavities are resealed with a small patch welded in place, or other sealing methods as discussed previously. Solid samples may be placed in open cavities if required. These cavities may then be sealed by incorporating thermal or sonic welding techniques in applying the cover sheet.

The configuration of the tray allows optimum usage of the volume. A tray in an inverted position nests and interlocks with an upright tray resulting in relatively little unoccupied volume within a filled storage module (Figure 6-23), and good heat transfer area to the cooling surface. The specimen cavities are maintained as groups in tray form during transport to the orbiting workshop and the return to ground. The cavities need not be separated until the specimens are transferred to the modules used to ship samples to the appropriate analytical laboratories.

6.2.2.2 Storage Module

The storage module measures 20 by 20 by 26-inches and has a capacity of 56-specimen trays (2016-specimen cavities). Inner and outer aluminum shells provide structural rigidity while a two inch thickness of polyurethane Freon-filled foam provides the insulation characteristics required for storing refrigerated and frozen specimens. The cross sectional dimension of 20 by 20-inches is dictated by the diameter of the smallest hatch the module must pass through. (See Figure 6-25.) For the purposes of this report, the Apollo Command Module with a 30-inch diameter hatch is considered as a likely ferry vehicle. Each corner of the transport module clears this hatch by one inch.

A hinged door assembly is used for interior access and provides a seal of maximum efficiency. Carrying handles and the door latch are recessed to provide a flush surface for efficient storage of the modules. Trolley attachment fittings for transportation are similarly recessed into the top surface of the module. The door latch is of the type that requires two grips to be pressed together. Since only compressive forces are involved the crew member does not tend to push himself away or pull himself toward the door as would be occurring with other latch types in zero-g conditions.

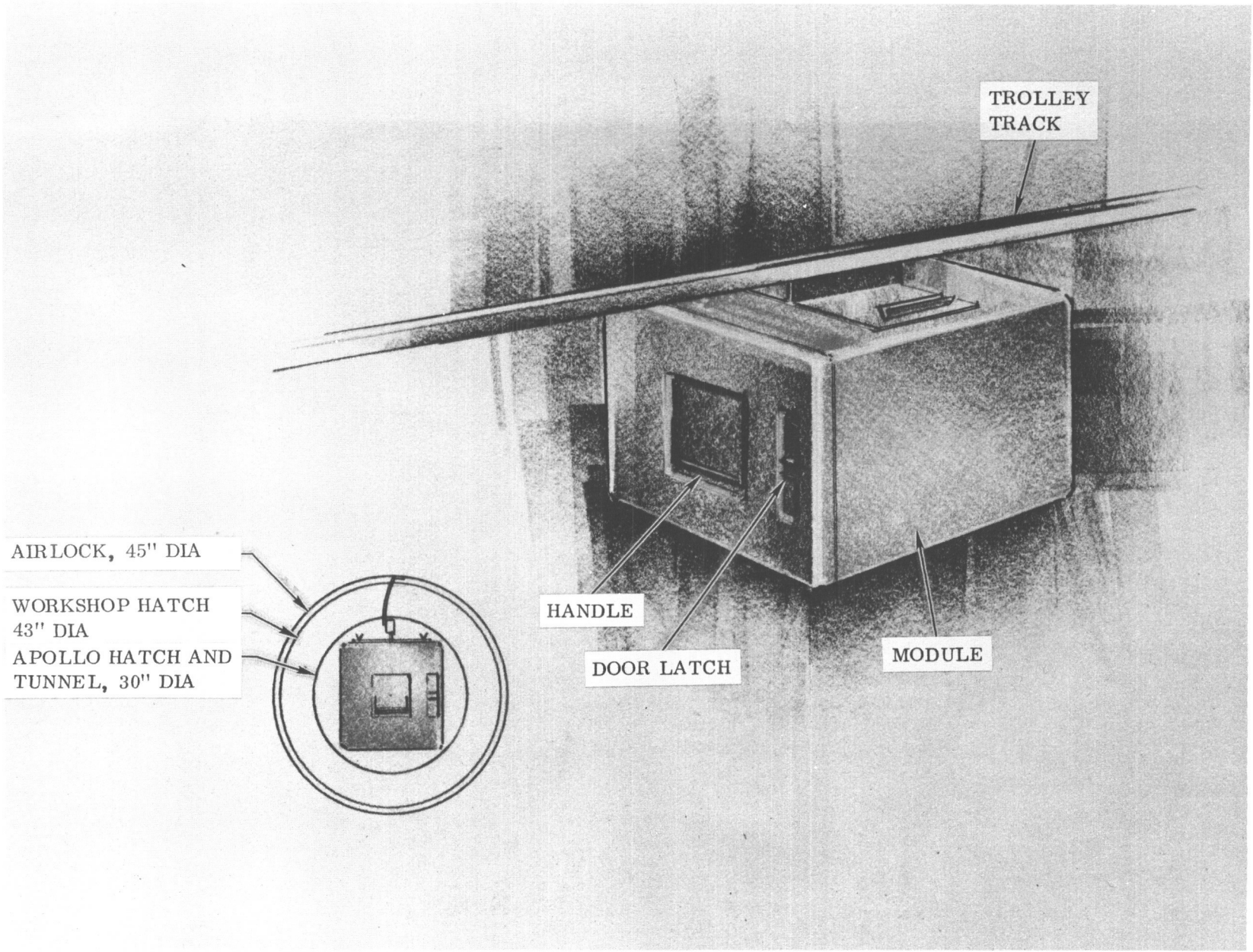

Figure 6-25. Sample Storage and Transportation Module-Design Concept

The interior configuration of the module features six shelves supporting the specimen trays. Three types of shelves are used. One type utilizes two plates with springs mounted to the opposing inside surface to exert an outward force, thereby retaining the specimen trays against the cooling surface. The other type includes refrigerant transport tubes to cool the plates. These two types of shelves are interchangeable and can be varied to provide optimum temperature conditions for the type of specimen being transported. (See Figure 6-26.) A third shelf type incorporates insulation material similar to that used in the walls of the module and may be used to separate the interior into compartments of different temperatures. Quick disconnect fittings recessed into the back surface of the container connect the cooling shelves to a refrigeration system aboard the ferry vehicle, workshop, or ground transport vehicle. In the case of freezing, the Freon vapor cycle unit will be attached to and shipped with the storage container. Thus, just electrical and condenser coolant flow ($60^{o}F$, $15.5^{o}C$) connections must be made to maintain sample temperature. The compressor of the vapor cycle unit will have a limited life and therefore may need refurbishment, thus it is returned with the storage module.

A refrigerated box of 1000-10 grams samples ($35^{o}F$, $2^{o}C$) has a heat leakage of 11.1 watts or 38 BTU/hour. Assuming that the affected thermal mass of the box is 25-pounds with a specific heat of 1 BTU/hr^{o}F, then the sample temperature will increase at a rate of approximately $1.5^{o}F$ per hour in a $75^{o}F$ ambient environment if no cooling is applied.

$$Q = WCp\,\Delta t$$
$$38 = 25 \times 1\,\Delta t$$
$$\Delta t = 1.5^{o}F$$

The rate of temperature increase will be reduced as the temperature of the box increases.

A freeze and storage box of 1000-10 gram samples ($-40^{o}F$) has a heat leakage of 48-watts or 164 BTU/hr. Assuming that the affected thermal mass of the box is 25-pounds with a specific heat of 0.5 BTU/hr^{o}F, then the sample temperature will increase at a rate of approximately $13.1^{o}F$ per hour in a $75^{o}F$ ambient environment if no cooling is provided.

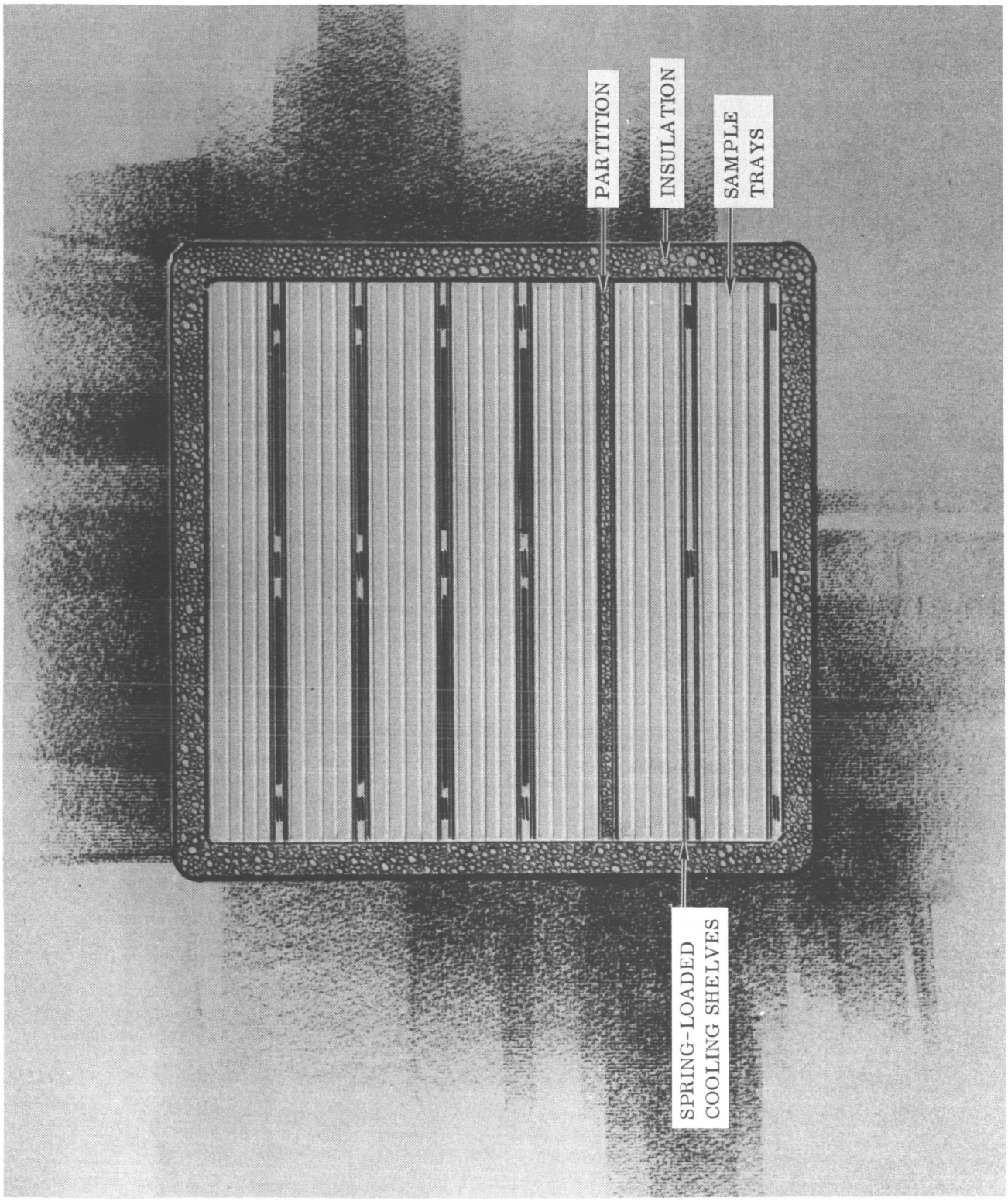

Figure 6-26. Sample Storage Module-Design Concept

$$Q = WCp\,\Delta t$$

$$164 = 25 \times 0.5 \times \Delta t$$

$$\Delta t = 13.1^{\circ}F$$

The rate of temperature increase will be reduced as the temperature of the box increases. Also, the change of phase from solid to liquid requires approximately 144 BTU/lb. At the above heat leakage rate of 164 BTU/hr, and 22-pounds of sample, it will require an additional 19.3-hours to completely melt the samples.

$$\frac{22 \text{ lb} \times 144 \text{ BTU/lb}}{164 \text{ BTU/hr}} = 19.3 \text{ hr}$$

Normally the ferry vehicle will have a space radiator and a water boiler for vehicle heat dissipation; however, it will not have an active temperature control mechanism during reentry and the several hours possible for recovery. It may be necessary, especially for the frozen samples, to provide an auxiliary means of maintaining temperature since there may not be sufficient electrical power to operate the Freon-vapor cycle unit and also a heat sink for the unit may not be available during recovery. A possible solution is to use an expendable refrigerant such as Freon 22 which will, at one atmosphere pressure, boil at $-41.4^{\circ}F$ ($-40.8^{\circ}C$) and maintain that temperature in the storage module until the Freon is depleted. The heat of vaporization of Freon 22 is 100.66 BTU/lb; therefore, the heat leakage of 164 BTU/hr into the freeze and storage box will consume $\frac{164 \text{ BTU/hr}}{100.66 \text{ BTU/lb}} =$ 1.64 lb of Freon per hour to maintain the freezer temperature.

Obviously, a thorough study of the ferry vehicle mission profile and vehicle design is required before a definitive design of the transportation and storage module can be completed. However, as discussed in this section, there is no problem which can not be solved with existing knowledge, and some flight hardware of this type has been and is being built.

6.2.3 TRANSPORTATION

During mission resupply, a ferry vehicle would attach to the orbiting workshop airlock and supplies could be transferred between vehicles via a trolley track device. The trolley track would be hinged out from storage in the air-lock and additional track sections would be added so that the sample storage module could be easily moved through the airlocks via a trolley track device. The biological samples would then be transferred to the ferry vehicle and return to earth for analysis. (See Figures 6-27 and 6-28.) The track extensions are easily attached and contain a stop and an antibacklash device to control the module at the end of travel.

The physical size of the module containing biological samples may be determined from the discussion of preservation techniques in Section 5. The storage capacity within the ferry vehicle may then be determined once the sampling regimen and mission profile are known.

6.2.4 TRASH DISPOSAL

A large amount of trash is produced in any space mission and presents a significant problem. In a space mission where large numbers of biological specimens are collected, the trash problem may be overwhelming. This is caused by the large numbers of required disposable syringes, sterile envelopes, swabs, collection devices, etc. To complicate the problem, some of the trash will be highly contaminated and may become dangerous to the crew. Consequently, the trash must be disposed of in an efficient and sanitary manner.

Since most of the trash is thermoplastic material, it is possible to compress the trash in a heated baler and store the trash in a minimal size bundle. The heating also sterilizes the trash and evaporates and jettisons the volatiles (residual water, etc.) to space. The baled trash must then be stored in a sterile sealed container to prevent recontamination.

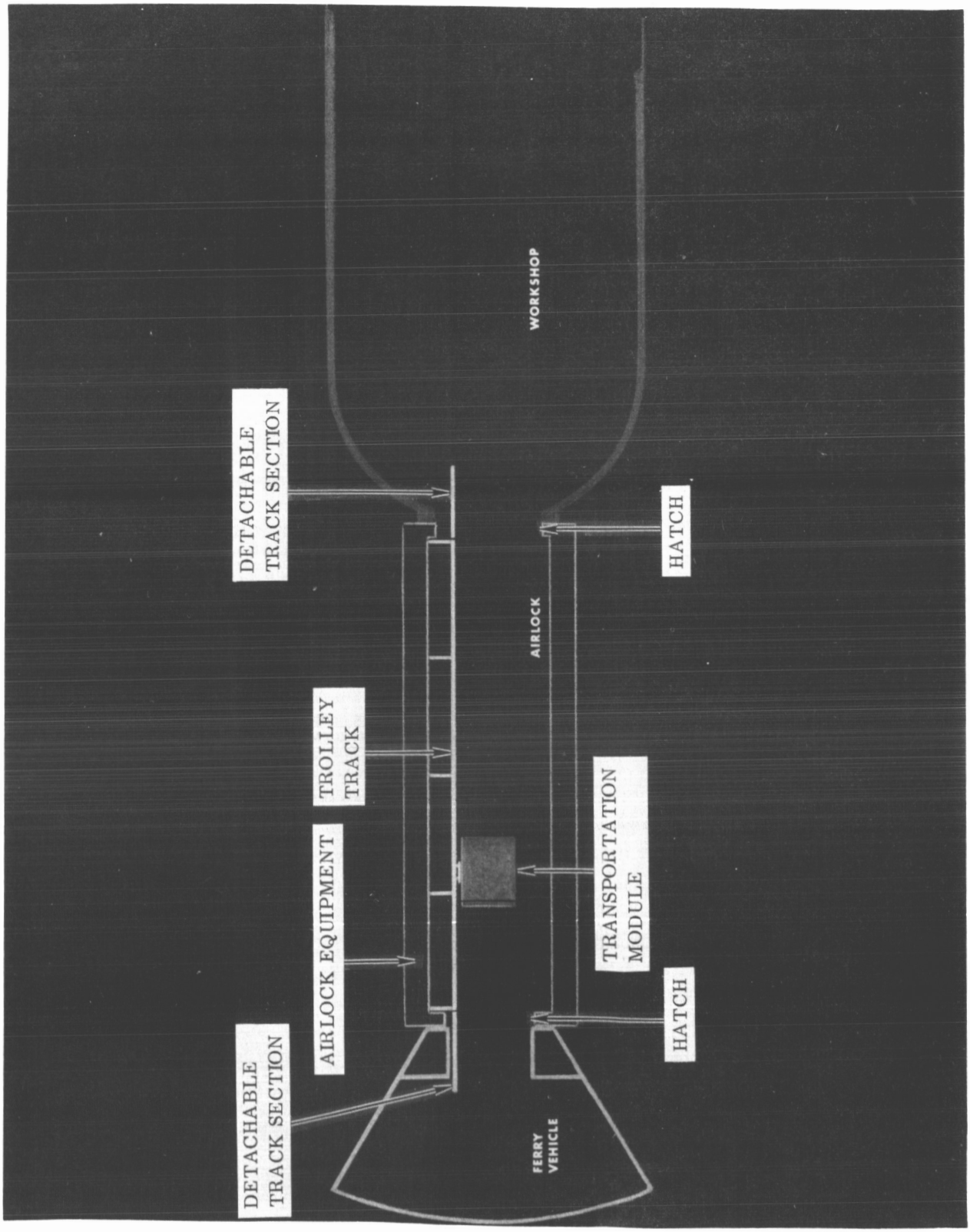

Figure 6-27. Airlock Layout

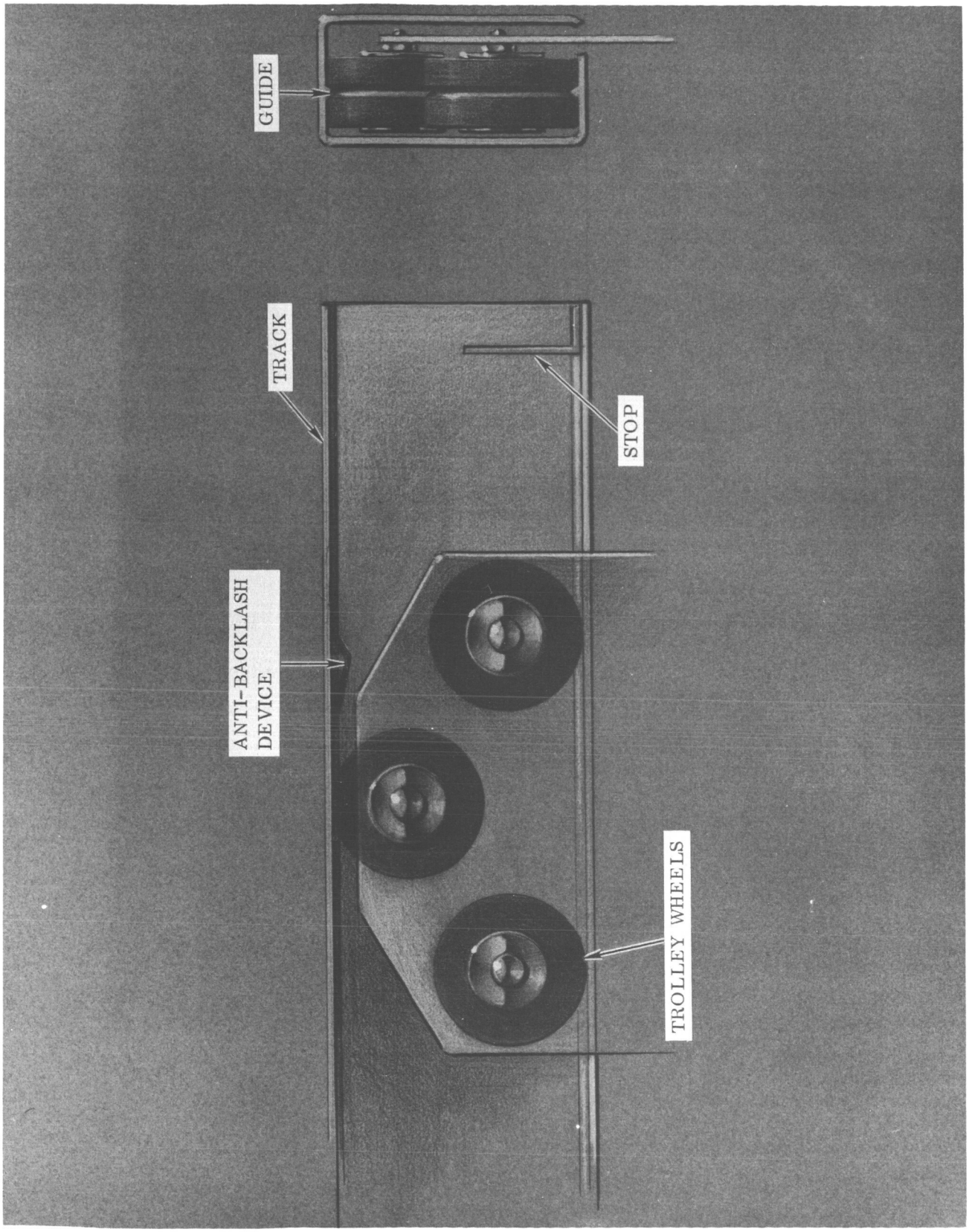

Figure 6-28. Trolley and Track for Module Transportation

A more positive solution to the trash problem is to incinerate it and jettison the resulting sterile gases and ashes to space. The decomposed trash is mainly carbon, hydrogen, and oxygen. Incineration requires oxidation of the hydrocarbons at approximately 700°F (371°C) in the following assumed reactions:

$$C + O_2 \rightarrow CO_2$$

$$2H_2 + O_2 \rightarrow 2H_2O$$

Approximately 3.3-pounds of oxygen are required to oxidize each pound of trash; however, it is not completely efficient. Therefore, it is assumed that five pounds of oxygen are required to incinerate each pound of trash. This is a significant weight penalty and will probably eliminate incineration from further consideration. A trash baler and sterilizer is recommended for trash disposal. (See Figure 6-29.)

The trash is collected in a container which is sealed with a piston. Heat is applied (about 165°C or 330°F) from electrical, radioisotope or waste heat sources and the container is vented to vacuum. The heat and the cabin atmospheric pressure on the piston causes the thermoplastic trash to collapse into a minimal volume. A cabin pressure of 5 psia will apply a force of nearly 400-pounds on a 10-inch diameter piston. The trash is sterilized and the volatiles evaporated to space. Approximately two hours will be required to sterilize the trash by dry heat. This is more than enough time for the thermoplastic material to collapse into a minimum size bundle. The container and trash is then cooled, ejected from the baler into a sterile sealable bag and is stored. The trash bale will have a fairly stable shape since the thermoplastic is cooled while in the compressed configuration.

6.3 BIBLIOGRAPHY

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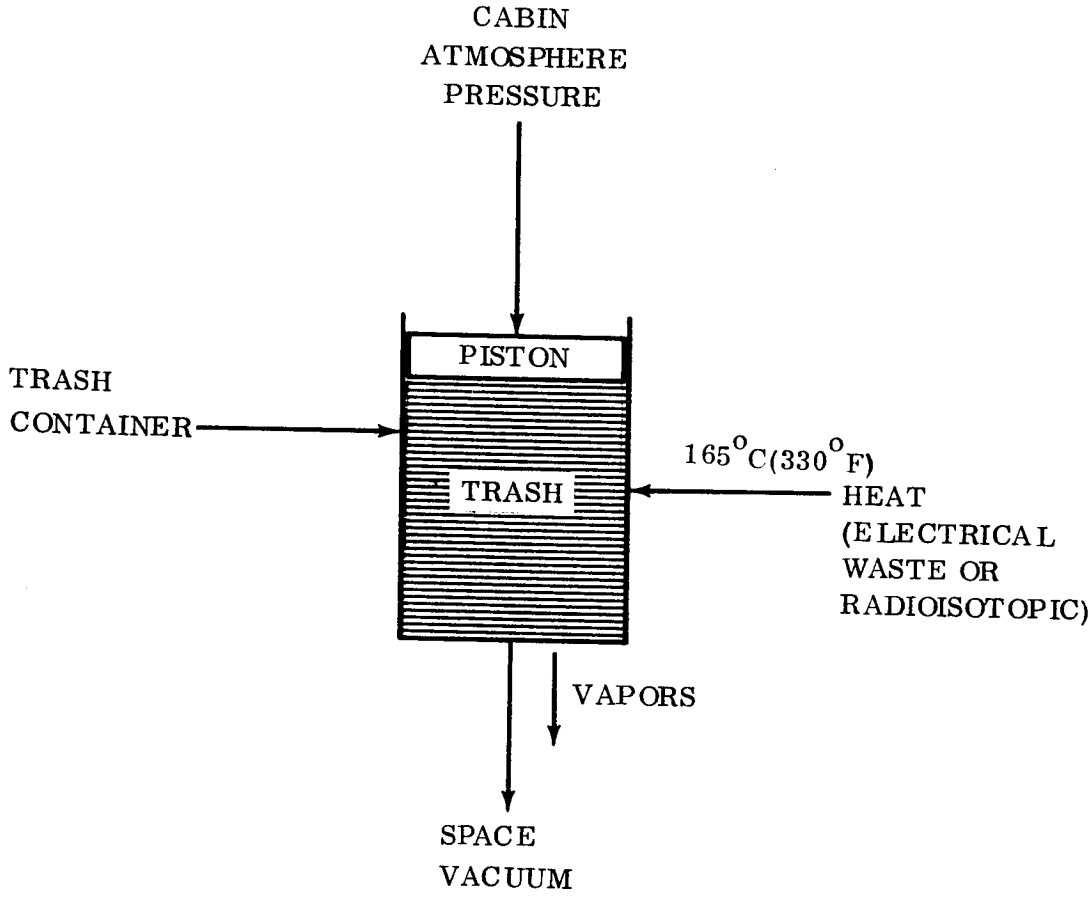

Figure 6-29. Trash Disposal Unit-Bailer Type

2. Reville, J. J., Murray, R. W., Cooper, L. Waste Collection and Sampling Assembly. General Electric Company, Contract P. O. No. 533205, November 1966.

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SECTION 7

SAMPLING REGIMEN

Either real time data or post facto time course plots require that some number of samples be taken. Ideally for each crew member, blood, urine, sweat and possibly parotid fluid samples would be taken several times each day and a stool sample at each defecation (assuming 1 or <1 defecation/day) regardless of which analyses are performed on-board and which post-flight. As we back off from an optimum situation, biologically speaking, the question arises as to what constitutes a minimum acceptable number of samples and on what schedule these samples should be taken. The scheduling is the simpler question to answer. Regardless of the number of samples taken, each sample should be obtained at the same time of each 24-hour period. Blood chemistry values are highly variable with the time of day; lowest values, often in a range heretofore considered pathologic usually occur in the evening although blood creatinines were highest in the early morning hours. For example, phosphate averages of 5-6 mg % at 8 a.m. and 3 mg % at 11 a.m. have been reported.

Since each astronaut will serve as his own control and the control values will be determined during pre-flight ground baseline studies, the crew work-rest cycles should parallel the 24-hour earth day for the close time correlation required. Furthermore, the baseline studies should be run both under the conditions of simulated flight, including confinement for a time period equal to the mission length, and during normal ground operation type working conditions.

The greater the number of samples obtained during the mission, the more meaningful the resultant data will be. There is no magic minimum acceptable number which will guarantee data of a certain confidence level. While one sample each of blood, urine, feces, and sweat is better than no sample at all, the data yield would certainly not be appreciable. Of major interest, physiologically, is the adaptation of man to a new environmental parameter, weightlessness. With more data points available, life scientists will be able to furnish better criteria for weightlessness countermeasures required, a regimen of personal hygiene, and probably better design criteria for crew equipment.

Assuming that all of the tests listed in Appendix A will be performed on samples obtained from each astronaut each day, Table 7-1 lists the total samples of urine, feces, and sweat required by astronaut, by day and for a flight of 45 days. The sample size has been estimated to permit duplicate assays for each test required.

The preferred method of preservation for urine is freezing; for feces and sweat, chemical preservation would be the method of choice. Since each of the three biological products would be sampled each day in the same amount, no complicated sampling or handling protocol would be necessary.

Table 7-1. Total Weights and Volumes, Tests in Appendix A, Duplicate Analyses*

	Total/Astronaut Per Day (Avg.)	Total Per Day (Avg.)**	Total/45-Day Flight**	Preservation
Urine	45 - 60 ml	135 - 180 ml	6075 - 8100 ml	Freezing
Feces	10 gms	30 gms	1350 gms	Chemical
Sweat	2.6 - 4.4 ml	7.8 - 13.2 ml	351 - 594 ml	Chemical
Serum or Plasma	10 ml	30 ml	1350 ml	Freezing
Whole Blood	3 - 4 ml***	9 - 12 ml***	405 - 540 ml***	Chemical or Freezing

* Urine, feces, and sweat - 1 sample/day; blood sampled as per 7-day rotating schedule.

** Assumes a 3-man crew

*** Does not include large samples of blood used for certain tentative on-board analyses.

A difficulty with this simple sampling protocol is that each astronaut may not defecate every day. In such a case, a sample of the same size (10 gms) would be stored for assay from each defecation when it did occur. (Feces stored using this protocol would be used for assaying inorganic ions only and not microorganisms.)

Again, assuming that all tests in Appendix A are performed, a seven day rotating schedule for each astronaut is proposed for withdrawing aliquots of blood. This is necessitated by the large volume of blood required to do all the tests and also by the fact that for some tests blood must be treated differently than the way it is treated for others. For example, some tests call for serum, others for citrated plasma, others for oxalated plasma and still others for whole blood. A further complication is that all tests for whole blood do not require similar preparation.

Table 7-1 lists the estimated total volumes of blood, serum, and plasma which would be required to do all of the tests listed in Appendix A on a rotating schedule described in Tables 7-2, 7-3 and 7-4. It should be noted that in the cases of plasma and serum volumes, it would be necessary to withdraw approximately twice as much whole blood to obtain a given volume. For example, if 1 ml of serum is required, 2 ml of blood would have to be withdrawn. This fact cannot be emphasized too strongly. Although it may appear from these tables that only moderate quantities of fluid are required, in actual practice relatively large blood volumes will have to be withdrawn. Every milliliter of serum or plasma required means that two milliliters of whole blood must be drawn because approximately half the total volume is comprised of formed elements.

Tables 7-1 and 7-5 assume a flight crew of three (3) men with either a 45-day flight or a 45-day resupply period. Table 7-1 lists the total volumes and weights of biological samples to be stored per man per day, (three times the former), and for a total period of 45 days (45 times the per day amount). Table 7-5 lists the engineering penalties in terms of weight, power, and volume required for storage and preservation of these samples by means of the methods of choice for a flight or resupply period of 45 days.

Table 7-2. Blood Sampling Protocol for Each Astronaut, 7-Day Rotating Schedule

(To be repeated every 7 days)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Serum	15 ml*	10 ml*	10 ml*	15 ml*			
Citrated Plasma					10 ml*		
Oxalated Plasma						5 ml*	
Whole Blood	1.2 ml	5.6 ml	3.4-7.6 ml	1.2 ml	5.4-7.6 ml	21.2 ml	33.2 ml

*For each milliliter of plasma or serum, 2 ml of whole blood must be withdrawn.

The blood sampling regimen of Tables 7-2, 7-3 and 7-4 is based on a seven day cycle as stated above. A shorter cycle period would require that much larger samples of blood be withdrawn each day. As it is, 20 to 30 ml of blood are withdrawn each day from each man.

Several things about these tables are worthy of especial note. First of all, over a period of 45 days, it can be calculated that 1.035 to 1.080 liters of blood will be withdrawn from each astronaut. This much blood is required just to do most assays only once a week. Secondly, the sampling protocol is extremely complicated and would require more than one venipuncture and capillary puncture on each of several days in the 7-day rotating schedule. Lastly, some of the tests (e.g., catecholamines) require disproportionately large blood samples for the amount of information obtained from the tests. Weights, powers, and volumes for storage are shown in Table 7-5. For these reasons, it might be well to resort to a simpler sampling regimen on initial flights which would omit certain tests but would still yield a great deal of information about the physiology of the astronauts. Such a protocol is described below.

Table 7-3. Sampling Schedule

(Maximum Number of Samples)[1]

Urine[2]	54.2 - 70.4 ml (gm)	Same size sample withdrawn each day throughout period.													CYCLE IS REPEATED	DAY DEPENDS ON N
Sweat[2]	2.6 - 4.4 ml (gm)	Same size sample withdrawn each day throughout period.														
Feces[2]	10 gms (ml)	Same size sample withdrawn each day throughout period.														
Whole Blood, Plasma and Serum[3]	Day 1 of rotating blood sampling schedule	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
	17.2 ml* (gm)	15.6 ml (gm)	13.4 - 17.6 ml (gm)	17.2 ml (gm)	20.4 - 22.6 ml (gm)	6.2 ml (gm)	1.2 ml (gm)	17.2 ml (gm)	15.6 ml (gm)	13.4 - 17.6 ml (gm)	17.2 ml (gm)	15.4 - 17.6 ml (gm)	6.2 ml (gm)	1.2 ml (gm)		
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14		N

*Actual volume of whole blood withdrawn each day varies from 20 to 35 ml. Volumes of fluid stated are those which must be stored during flight.

1. The assumption is made that 1 gm = 1 ml for all specimens. Thus, whether the quantity withdrawn is normally expressed in volumes or weights the numerical value is approximately the same for both.

2. Samples required from each astronaut to perform duplicate analyses for parameters in Appendix A.

3. For description of tests to be performed in duplicate on each sample and rotating schedule, see Tables 7-2 and 7-4.

N. B. This schedule can be used to cover preflight, orbital and postflight periods.

Table 7-4. Blood Sampling Regimen

Tests to be performed in duplicate on samples withdrawn, seven-day rotating schedule in detail

Day Sample Withdrawn	Test		Fluid	Volume	Preservation
1 - 7	Reticulocyte Count WBC Differential Platelet Count		Whole Blood	0.6 ml	Chemical
1 - 7	RBC (Total) WBC (Total)		Whole Blood	0.4 ml	Chemical
1 - 7	Hematocrit		Whole Blood	0.2 ml	On-Board Determination
1	Catecholamines		Serum	10 ml*	Freezing
1	Glucose Tolerance Test		Serum	5 ml+	Freezing
2	Creatine Creatinine Serum Proteins Uric Acid Potassium Chlorides Phosphates Alkaline Phosphatase Calcium	Sulfates BUN Sodium Bilirubin PBI Thyroxine Magnesium Manganese Zinc Bicarbonate	Serum	10 ml	Freezing
2	RBC Cell Mass RBC Survival		Whole Blood	4 ml	Freezing
2	Hemoglobin Methemoglobin		Whole Blood	0.4 ml	Freezing hemolyzed dilution
3	Mucoproteins NPN Plasma Volume		Serum	10 ml	Freezing

+Includes several samples required for test.
*Single assay only.

Table 7-4. Blood Sampling Regimen (Cont.)

Tests to be performed in duplicate on samples withdrawn, seven-day rotating schedule in detail

Day Sample Withdrawn	Test	Fluid	Volume	Preservation
	Immunoglobulins LDH Isozymes Transferrins			
3	Blood Lactic Acid	Whole Blood	1 - 3 ml	Freezing protein precipitated solution
3	Karyotyping	Whole Blood	0.2 - 0.4 ml	On-board Determination
4	TBPA Immune Bodies	Serum	10 ml(?)	Freezing
4	Fat Tolerance Test	Serum	5 ml+	Freezing
5	Amino Nitrogen Fibrinolytic Activity AHG ADH ACTH	Citrated Plasma	15 ml(?)	Freezing
5	Clotting Time	Whole Blood	0.2 - 0.4 ml	On-board Determination
5	Clot Retraction	Whole Blood	4 - 6 ml	On-board Determination
6	Fibrinogen Prothrombin Activity PTC	Oxa-lated Plasma	5 ml	Freezing
6	WBC Motility and Phag. Act.	Whole Blood	20 ml*	On-board Determination

+Includes several samples required for test.
*Single assay only.

Table 7-4. Blood Sampling Regimen (Cont.)

Tests to be performed in duplicate on samples withdrawn, seven-day rotating schedule in detail

Day Sample Withdrawn	Test	Fluid	Volume	Preservation
7	Platelet Adhesiveness	Whole Blood	32 ml	Onboard Determination

Table 7-5. Approximate Weight, Power, and Volume to Preserve Samples for Appendix A by Methods Chosen

(See Table 7-1)

	Weight (lb)	Power (watts)	Volume (cu ft)
Urine	9.4	7.0	1.4
Feces	0.42	0	0.0530
Sweat	0.13	0	0.0177
Serum or Plasma	5.8	2.3	0.5
Whole Blood	0.13 - 5.3	0 - 1.1	0.0177 - 0.4
Total	17.9 - 23.1	9.3 - 10.4	1.99 - 2.37

The volumes of biological fluids which would have to be stored would be slightly larger than those required for subsequent analysis because of the problem of loss of fluid due to adherence to the sides of storage containers, pipettes, syringes, etc. Rough estimates have been made of such loss and have been figured into the amounts listed in these tables (especially with reference to blood, serum, and plasma). Measurement of actual volume loss because of this would require laboratory investigation utilizing these rotating schedules or some others which may be finally accepted. For example, on "Day 2" of the rotating

blood schedule, 20 analyses are required upon one 10 ml sample of serum (Table 7-4). At the earth-based laboratory, every time an aliquot of this sample is pipetted (forty times for 20 tests in duplicate), some serum will be lost which adheres to the sides of the pipette. Then too, some will be lost by adhering to the sides of the storage container. However, only 7.3 ml of serum are required for all 20 analyses. This allows 2.7 ml leeway for these and other losses. Whether this much, more, or less is required for such losses would require experimental verification in all cases. On the other hand, a cycle of longer duration would mean that the periods between subsequent assays of the same constituent would be increased. This would also increase the chance of missing important variations in these constituents of interest. The ideal case would be that enough blood be withdrawn every day to do every test. But this would mean an average daily blood sample of about 140-150 ml per man to allow for analysis in duplicate. Such a sample would be equivalent to mild hemorrhage and might well jeopardize the health of the astronauts if repeated over a long period of time.

All tests are not of equal importance. It is, unfortunately, impossible at this point in time to rank the various clinical assays on a completely objective basis. However, the hematological and biochemical measurements of interest can be grouped and the groups ranked according to their relative physiological importance.

The measurements in Appendix A range from common clinical tests, which are done by the thousands each day, to relatively esoteric methods which are performed by specially trained research technicians in a small number of institutions. While each measurement aids in depicting the physiological state of an individual, the contribution of about 1/5 of the tests considered to the total picture outweighs the data derived from the other 4/5. Much of the information derived from the larger group would be essentially corroborative in nature. For the purposes of the AAP missions, the tests which could be included in the experiments can be divided into four categories, as follows:

Group I - Feasible; requiring small sample; most important in light of present theory or experience related to possible physiological problems in space.

Group II - Feasible; important; should be done if at all possible.

Group III - of interest; but because of size of sample, difficulty of test, smaller likelihood of change in the value, or because test(s) in (1) and (2) might give the same information, these tests are accorded lower priority.

Group IV - Of limited interest; require large sample, difficult to perform, require a specific special study

Although various clinicians and physiologists may differ on some details of the categorization, a decision should be made by NASA, based on the advice of the scientific community, on the relative importance of the clinical tests and evaluations to be performed on samples of biological materials collected inflight and priorities established.

The relative importance of any given test should be determined by:

a. The physiological significance of that test per se.

b. The physiological significance of any given test complemented by small number (2-4) other tests.

c. The priority assigned to the experiment(s) which may include a given test in a protocol based upon a rigorously developed rationale.

The final priority assignment should result from a trade-off between two factors: an original priority assignment based solely on scientific merit (a NASA/scientific community decision) and a feasibility ranking, based on flight schedules, flight qualified hardware lead time, and crew skills required (a NASA/industrial community decision).

The large number of experiments proposed for the AAP flights has been winnowed down to 23, but these require performance of approximately 90 laboratory procedures. Even within

the confines of this study, which is mainly concerned with the in-flight preservation of samples for post-flight analyses, it should be evident that simply in order to obtain the necessary amounts of whole blood, plasma, and serum required on the schedule shown in Table 7-2, a tremendous amount of time and expendable supplies will be used. It would seem wiser to start with a minimum complement of analyses, meeting the requisites of the above-stated Group I definition and gradually adding to the test array as equipment is developed, modified laboratory methods are standardized, and in-flight procedures are proven. A series of assays meeting the criteria of Groups I through IV are given in Table 7-6. The collection, handling, and preservation problems are also approached in stepwise fashion as can be seen from Tables 7-8 through 7-11.

A number of assays, not included in Appendix A, have been suggested for addition here and are listed separately in Table 7-7.

The absence of a number of the assays as listed in Appendix A is intentional. Platelet adhesiveness, for example, is by no stretch of the imagination a quantitative test, requires an enormous amount of whole blood (an already large margin of error is magnified by smaller samples), is not feasible to perform on preserved samples, and does not really add much to our understanding of man's physiology during weightlessness. Other candidates for omission are listed in Table 7-12.

Subsequent inclusions of any or all of these assays in a post-flight scheme of analyses should be dependent upon proof of physiological significance, reliable standards obtained on preserved blood, and methods of proven precision.

As an example of what can be done with samples of approximately 10 ml, assuming that standards for analyses on preserved samples will be available, Table 7-13 was prepared.

The old bromide "You have to crawl before you can walk" should be kept in mind when the sampling regimens and complement of analyses for the AAP flights are generated.

Table 7-6. Ranked Groups of Clinical Evaluations

Group I		Volume	Preservation Method
Blood			
Whole	Hemoglobin	0.1	Freeze (hemolyzed sample)
	Hematocrit	0.1	On-board
Serum	Calcium	0.5	Freeze
	Potassium	0.1	
	Sodium	0.1	
	Chloride	0.1	
Urine			
	Sodium	0.2	Freeze
	Calcium	0.5	
	Potassium	0.2	
	Phosphorus*	1.0	
	Creatinine	0.1	
	Hydroxyproline*	2.0	
	Volume	---	Automatic, on-board
	Color*	---	---
Feces			
	Chloride	1.0	Chemical, chemical and refrigeration, lyophilization, or freeze
	Calcium	1.0	
	Potassium	1.0	
	Sodium	1.0	
	Color*	---	On-board
Sweat	Sodium	0.1	Chemical, chemical and refrigeration, or freeze
	Potassium	0.1	
	Chloride	1.0	
	Calcium	0.5	

* Assays not included in Appendix A

Table 7-6. Ranked Groups of Clinical Evaluations (Cont.)

Group II		Volume	Preservation Method
Blood			
Whole		---	---
Serum	Glucose	0.1	Freeze
	Total Protein	0.1	
	Protein Fractions		
	Albumins*	0.1	
	Globulins*	0.1	
	Creatine	0.2	
	Creatinine	0.2	
	Phosphorus	0.1	
	Cholesterol	0.2	
	Urea N	0.1	
	Lactic Dehydrogenase	0.2	
Urine			
	Chloride	0.5	Freeze
	Creatinine	0.1	
	Urobilinogen*	1.0	
	Indican*	1.0	
	pH (approximate)	---	---
	Urea N	0.2	Freeze
	Total N	0.2	
	Albumin	0.1	
	Mucoprotein	0.2	
Feces			
	Total N	0.3	Chemical, chemical and refrigeration, or freeze
Group III			
Blood			
Whole	(Deproteinize) Lactate/Pyruvate*	0.2	Freeze

* Assays not included in Appendix A

Table 7-6. Ranked Groups of Clinical Evaluations (Cont.)

Group III (continued)		Volume	Preservation Method
Plasma	(Oxalated) Fatty Acids*	2.0	Freeze
Serum	Magnesium	0.1	Chemical, refrigeration or freeze
	Lipase*	0.1	Freeze
	Alkaline phosphatase	0.1	Freeze
	Phospholipids*	1.0	Freeze
	Protein bound iodine	0.2	Freeze
	Uric Acid	0.2	Freeze
	Iron*	0.2	Chemical, refrigeration or freeze
Urine			
	Uric Acid*	1.0	Freeze
	Amino Acids*		
	Qual.	1.0	Freeze
	Quant.	10.0	Freeze
	Magnesium	0.2	Chemical, refrigeration or freeze
	5-Hydroxyindolacetic Acid*	1.0	Freeze
Feces			
	Total fat and partitions*	3.0	Freeze
Group IV			
Blood			
Plasma	(Heparinized)*	5-10	Freeze
	Cortisol (C^{14})		Freeze
	Circulating ACTH*	0.5	Freeze

* Assays not included in Appendix A

Table 7-6. Ranked Groups of Clinical Evaluations (Cont.)

Group IV (continued)		Volume	Preservation Method
Serum	Parathyroid peptides*	Unknown	Freeze
	Catecholamines	10	
	Insulin*	5	
	Triglycerides*	1.0	
Urine			
	17-hydroxycorticosteroids	5.0	Freeze
	17-ketosteroids*	5-50	
	Tetrahydroaldosterone*	10-30	
	Aldosterone	15	
	Catecholamines*	25	
	Testosterone*	300-500	
	Manganese	0.1	Chemical, refrigeration or freeze
	Pyrophosphate*	1-5	Freeze
Feces			
	Mg*	0.5	Chemical, refrigeration or freeze
	Trypsin*	0.2	Freeze

*Assays not included in Appendix A

Table 7-7. Significance of Assays in Groups I-IV
(Not Listed in Appendix A)

Group I		
Urine	Phosphorus, Hydroxyproline	Relevant to dissolution of bone matrix.
	Color	Color chart for comparison; no weight, power, or volume cost for information.
Feces	Color	Gross appearance of clinical interest.
Group II		
Serum	Protein fractions	Relevant to state of hydration, muscle activity.
	Cholesterol	Relevant to acute infections, thyroid functions.
Urine	Urobilinogen	Relevant to diarrhea, self-administered oral antibiotics.
	Indican	Relevant to constipation, diet protein level, abscesses.
Feces	Total N	Indicator of nitrogen balance.
Group III		
Whole Blood (deproteinize)	Lactate/Pyruvate	Indicator of muscle activity.
Plasma (oxalated)	Fatty Acids	Relevant to thyroid function.
Serum	Lipase	Indicator of lipolytic activity.
	Phospholipids	Indicator of pancreatic function.
	Iron	Indirect check on hemoglobin.
Urine	Uric Acid	Indicator of muscle atrophy
	Amino Acids	Reflect changes in diet and/or metabolism.
	5-Hydroxyindolacetic Acid	Indirect check on serotonin level.
Feces	Fat and partitions	Levels dependent on dietary fat and bowel activity.
Group IV		
	Mostly hormone assays which would give a clearer picture than 17-hydroxycorticosteroids and aldosterone only; parathyroid peptides should be of definite relevance to calcium balance.	

Table 7-8. Total Volumes (Weights)*

	Group I	Group II	Group III	Group IV
Whole Blood	0.4 ml	—	0.4 ml	—
Plasma	—	—	4.0 ml	11.0 - 21.0 ml
Serum	1.4 ml	2.6 ml	4.6 ml	32 ml
Urine	8.0 ml	6.6 ml	26.4 ml	722.2 - 1260.2 ml
Feces	8.0 ml	0.6 ml	6.0 ml	1.4 ml
Sweat	3.4 ml	—	—	—

* 1 ml = 1 gm Volumes (weights) required for analysis in duplicate

Table 7-9. Chemical Preservation*
(150 Samples)

	Group I			Group II			Group III			Group IV		
	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)
Whole Blood	0.0551	NO	0.00706	---	NO	---	0.0551	NO	0.00706	---	NO	---
Plasma	---	POWER	---	---	POWER	---	0.165	POWER	0.0247	0.441-0.826	POWER	0.0565-0.106
Serum	0.110		0.0141	0.121		0.0172	0.176		0.0264	1.32		0.176
Urine	0.386		0.0423	0.276		0.0363	1.10		0.141	29.8-50.1		3.81-6.46
Feces	0.386		0.0423	0.022		0.00883	0.274		0.0335	0.110		0.0141
Sweat	0.165		0.0212	---		---	---		---	---		---
Total	1.10		0.127	0.419		0.0623	1.77		0.233	31.7-52.4		4.06-6.76
Total Group I + II				1.52		0.189						
Total Groups I + II + III							3.29		0.422			
Total Groups I + II + III + IV										35.0-55.7		4.48-7.18

*Volumes, powers, and weights of packages necessary to store samples, 1 sample per astronaut per day for 50 days.

Table 7-10. Preservation by Refrigeration*
(150 Samples)

	Group I			Group II			Group III			Group IV		
	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)
Whole Blood	2.05	0.14	0.0220	---	---	---	2.05	0.14	0.0220	---	---	---
Plasma	---	---	---	---	---	---	2.44	0.46	0.0900	3.35-4.25	0.9-1.4	0.251-0.459
Serum	2.14	0.24	0.0424	2.25	0.35	0.0617	2.49	0.48	0.0960	5.60	1.8	0.677
Urine	3.00	0.7	0.180	2.69	0.6	0.159	4.80	1.5	0.705	48.5-80.0	15-22	15.9-25.9
Feces	3.00	0.7	0.180	2.09	0.16	0.0268	2.74	0.56	0.120	2.14	0.25	0.0424
Sweat	2.36	0.42	0.0796	---	---	---	---	---	---	---	---	---
Total	12.6	2.21	0.504	7.03	1.11	0.248	14.5	3.14	1.03	59.6-92.0	18.0-25.5	16.9-27.1
Total Groups I + II				19.6	3.32	0.752						
Total Groups I + II + III							34.1	6.46	1.78			
Total Groups I + II + III + IV										93.7-126	24.5-32.0	18.7-28.9

*Assuming a refrigeration temperature of approximately 35°F (2°C).

Table 7-11. Preservation by Freezing*
(150 Samples)

	Group I			Group II			Group III			Group IV		
	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)	Weight (lb)	Power (watts)	Volume (cu ft)
Whole Blood	4.98	0.35	0.0399	---	---	---	4.98	0.35	0.0399	---	---	---
Plasma	---	---	---	---	---	---	5.14	0.66	0.120	6.00-7.01	2.6-4.2	0.269-0.480
Serum	5.06	0.46	0.0600	5.12	1.2	0.0895	5.20	0.68	0.135	7.98	5.5	0.705
Urine	10.0	2.2	0.210	5.60	1.7	0.180	7.39	4.8	0.561	234-415	44-62	17.6-30.0
Feces	10.0	2.2	0.210	5.03	0.37	0.0494	5.55	1.6	0.169	5.07	0.46	0.0600
Sweat	5.20	1.3	0.109	---	---	---	---	---	---	---	---	---
Total	35.2	6.51	0.629	15.8	3.27	0.319	28.3	8.09	1.02	253-435	52.6-72.2	18.6-31.2
Total Groups I + II				51.0	9.78	0.948						
Total Groups I + II + III							79.3	17.9	1.97			
Total Groups I + II + III + IV										332-514	70.5-90.1	20.6-33.2

*Assuming a freezing temperature of approximately $-4^{\circ}F$ ($-20^{\circ}C$).

Table 7-12. Candidates for Omission From Preservation Regimen

Glucose tolerance test Fat tolerance test	Too many samples within a short period of time
Blood lactic acid	Susceptible to major technical error
Blood catecholamines	Represent transitory phenomena only
Thyroxine and TBPA	Difficult to measure; PBI may give sufficient information
RBC (total)	Difficult to preserve for meaningful results
Platelet count WBC motility and phagocytic activity. Platelet adhesiveness Clot retraction Karyotyping	Can only be done on fresh blood

Table 7-13. Duplicate Analyses from a 10 Gram* Sample

Urine+		Serum+	
Sodium	(0.3)	Sodium	(0.1)
Potassium	(0.3)	Potassium	(0.1)
Calcium	(0.3)	Calcium	(0.2)
Magnesium	(0.3)	Magnesium	(0.2)
Phosphorus	(2.0)	Phosphorus	(0.2)
Chloride	(1.0)	Chloride	(0.2)
Hydroxyproline	(4.0)	Creatine	(0.2)
Creatine	(0.2)	Creatinine	(0.2)
Creatinine	(0.2)	Protein Bound Iodine	(0.4)
Serotonin	(0.2)	Urea Nitrogen	(0.2)
Total Nitrogen	(0.4)	Non Protein Nitrogen	(0.2)
		Phospholipids	(2.0)
		Protein Fractions	(4.0)
		Glucose	(0.2)
		Bilirubin	(0.4)

* Assumed that 1 gm = 1 ml

+ Volumes total less than 10 ml to allow for fluid that adheres to sample tube and pipettes

SECTION 8
TRAINING

The most important element of a successful course of training in collection and preservation methods will be astronauts who are strongly motivated to assist the scientific and medical communities to obtain meaningful data during orbital flight concerning the various physiological changes which may occur with time. Since the S-IVB Laboratory is still in the future, it is hoped that the engineering aspects of orbital flight will have received sufficient attention to permit more time in orbit, and also more time on the ground in training, for physiological and biochemical procedures.

Overall crew training requirements will be governed by the presence or absence of a crew member with prior experience in clinical laboratory procedures. With the prcsent crew selection criteria, the most likely astronaut candidates who would have some applicable experience would be Flight Surgeons. These individuals, though, will still need considerable training because most of the blood-letting, urine and feces sample taking, sweat collecting and bacteria swabbing is, in practice, done by paramedical personnel, e.g., nurses and technicians. Familiarity with the procedures and the background necessary to appreciate their use are assumed to be implicit in the title Flight Surgeon.

Case 1: Flight Surgeon a crew member

The Flight Surgeon should be assigned prime responsibility for all collection and preservation procedures. His training would be essentially the same as that of other crew members, but during the training sessions he would act as a "laboratory instructor"; thus,by assuming a teaching role, he would improve his own performance. The more facile of the other two crew members should be assigned as back-up man.

Case 2: No crew member with prior clinical laboratory experience

The same training course would be used. However, a crewman assigned as back-up man in one crew would be assigned prime responsibility in another crew. He would, therefore, go

through the training twice. During the second time around he would act as "laboratory instructor" and another crew member would become the back-up man.

A training regimen need not involve separate formal classroom and laboratory sessions because of the small number of students. It is recommended that the classes be kept small and restricted to not more than two crews. An outline of the training program follows.

8.1 TRAINING PROGRAM OUTLINE

SUBJECT	CLOCK HOURS
Orientation	
1. Results of Pre- and Post-Flight Physiological and Biochemical Measurements on Astronauts.	3 Hours
2. Demonstrations of equipment and procedures used (e.g., ECG, urine sampling, tilt table, etc.)	6 Hours
3. Discussion of biological and medical experiments in which crew members will serve as subjects (why experiments are being performed, experimental design, etc.)	6 Hours
4. Demonstrations of equipment and procedures to be used by or on crew members.	12 Hours
Laboratory Procedures Training	
1. General (need for care, attention to detail, definitions, e.g., cleanliness vs. sterility, etc.)	3 Hours
2. Sterile Technic (what it is and why it is important)	3 Hours
3. Microbiological Sampling	6 Hours
Microbiological Sampling Review	2 Hours
4. Microbiological Culturing	4 Hours

	SUBJECT	CLOCK HOURS
5.	Microbiological Sample Handling	6 Hours
	Microbiological Sampling and Handling Review	3 Hours
6.	Collection of Blood (venipuncture, lancet)	3 Hours
7.	Handling of Blood (preparation of serum, plasma, for storage, preservation)	3 Hours
	Review of blood collection and handling	3 Hours
8.	Urine Collection and Handling	2 Hours
	Review of Blood Collection and Handling	1 Hour
9.	Sweat Collection and Handling	3 Hours
10.	Feces Collection and Handling	2 Hours
	Review of Blood Collection and Handling	1 Hour
	Flight Simulation	
1.	Experiment Protocol Discussion	3 Hours
2.	Run-thru of sample collection and handling required by flight experiment plan.	6 Hours
3.	Familiarization with flight experiment prototype equipment.	3 Hours
4.	Review of sample collection and handling procedures using prototype flight equipment	6 Hours
5.	Review, in lab module mockup, of sample collection and handling procedures using prototype collection,handling and preservation equipment	24 Hours
6.	Review, in flight simulator, of sample collection and handling procedures using flight-type equipment	26 Hours
	TOTAL	150 Hours

The microbiolobical aspects of the training are listed early in the course because this is a logical place to discuss sterile techniques and the consequences of a breach of sterility. It should then be easier for the student to grasp the reasons for aseptic collection of blood and to understand the reasons for the exercise of extreme care in obtaining samples of urine, feces, and sweat.

Insofar as possible, all samples collected should be subjected to analysis. The samples obtained during training can be used as controls and to establish a pre-flight baseline for each man.

SECTION 9

CONCLUSIONS AND RECOMMENDATIONS

After a four-month study of a multi-faceted problem containing dependent, independent, and confounding variables, it would be presumptuous to label the bulk of our conclusions as any thing other than "tentative". They are discussed below, as our recommendations for future study.

9.1 CONCLUSIONS

In a number of instances, a recommendation for work in a given area is the result of a conclusion that insufficient information is available.

9.1.1 ANALYTICAL METHODS

The analytical methods deemed best for each of the body constituents are described in Section 3. During the selection of these techniques, two suspicions were confirmed: 1) Precise, accurate, and sensitive tests of proven worth are readily available for elements, inorganic radicals, and relatively simple organic compounds; 2) for complex organic compounds ranging from steroids to proteins, precision and/or accuracy tend to diminish rapidly. Another drawback to many such tests is the large sample volume required.

Clinical tests on whole blood are for the most part approximations which are compared to an empirically obtained set of standards. A common determination such as a WBC differential is subject to considerable fluctuation even when done by the same technician on two samples of blood taken within a few minutes of each other from one person.

Platelet counts can and do vary to the extent of $\pm$ 35%. While such tests do supply qualitative information, their lack of precision and accuracy place them low on the priority list for inclusion in a set of flight experiments.

Urine analyses, with the exception of 17-hydroxycorticosteroids, aldosterone, serotonin, and vasopressin, can be done within the precision limits, set by NASA, of $\pm$ 5%. This is in keeping with the statement above regarding loss of precision in tests for the more complex organic compounds.

Feces and sweat electrolytes can be measured with comparative ease.

9.1.2 MICROORGANISMS

Disagreement is rife among biologists dealing with "locked flora", and microorganisms indigenous to the skin and gut over what constitutes a "normal flora", how "normal" should be defined, how measurements of changes in skin and gut microfloral populations should be made, how samples should be taken and incubated, and the roles of protozoa and particularly viruses in man's personal microorganisms. The unequivocal statement can be made that neither the theory nor the practice of microbiology has yet developed to a state where accurate precise quantitative data on changes in skin and intestinal microorganisms can be obtained on the ground, much less in orbit. Qualitative data though can be obtained but must be carefully interpreted.

9.1.3 PRESERVATION

By far and away, chemical preservation is the simplest and most economical method with minimum weight and volume and no power cost. Unfortunately, electrolyte determinations are the only accurate assays that can be made on samples preserved in this fashion. The handful of reports in which various assays were made on stored samples, preserved chemically by freezing and refrigeration, have proven of minimal value during this study. There is no body of literature devoted to clinical analyses on preserved and stored samples. A considerable amount of work has been accomplished in the freezing of blood for future transfusions, but not only is most of the preservation at dry ice ($-78^{o}C$) or liquid nitrogen ($-195^{o}C$) temperatures, (prohibitively low to reach and maintain within the biomedical experiment weight and power bogies shown in Appendix C), but the criteria for efficacy of preservation are concerned with the attribute of cell viability, not minimal changes in value of blood constituents.

For the analyses listed in Appendix A, freezing, refrigeration, and incubation will all be required, plus chemical preservation. For purposes of safety, it may be necessary to refrigerate chemically preserved specimens.

Lyophilization and vacuum distillation, with the exception of reducing wash water volume, are feasible but are considered inferior. Lyophilization is an excellent technique for preserving many sample constituents, but engineering penalties are severe, and freezing preserves these constituents just as well for lfights of several months duration. Drying of samples over a desiccant looks good on paper, but no data on this method is available. Ion exchange resins, ring over chromatography, and other adsorption or absorption techniques are much too unwieldy for application to flight experiments.

9.1.4 COLLECTION AND HANDLING

Feces and urine can be collected automatically. There are no handling problems with the recommended systems.

Sweat, whether the collection bag or washing machine is used, will require a bit more effort on the part of the crew. Handling of the sample is quite straight forward.

Collection and handling of fecal samples, swabs, etc. for microorganism assay, though, is a different story. A highly motivated and well trained crew is mandatory if strict adherence to a sampling protocol is to be assured.

For proper collection and careful attention to the several blood handling schemes, a well trained and highly motivated crew is again mandatory.

Drawings of various pieces of apparatus for use during weightlessness are shown; all would require relatively short development times.

9.1.5 SAMPLING REGIMEN

The number of blood samples and their volumes, when taken on the 7-day rotating schedule (Table 7-2) which includes all the clinical evaluations in Appendix A, can be cut to a more reasonable number if those evaluations listed in Table 7-11 are omitted.

9.1.6 TRAINING

During a GE sponsored test, engineering personnel were trained within a week to draw blood from one another and did so for a month. During another company funded study, test pilots had a great deal of difficulty in using a spirometer. The difference between the two groups was not in intelligence or prior experience but in motivation; without a strongly motivated crew, the experiments will not be done properly, if at all.

9.1.7 RELIABILITY AS RELATED TO COMPLEXITY FROM THE BIOLOGICAL VIEWPOINT

In general the more complex a procedure is for collection, handling, or preservation, the less reliable. At every stage there is the possibility of operator error or system failure with resultant loss of sample and/or hazard to the astronauts. This is particularly true of some of the procedures necessary for handling blood samples. However, this problem has two sides to it. Some techniques and equipment may have to have complexity built-in to ensure greater reliability, especially with reference to stabilization of constituents. For example, serum or plasma has to be separated from blood for many analyses. In order to be able to do this in zero gravity, special containers must be developed and an on-board centrifuge will be necessary. A great deal of operator finesse will also be required. It would be desirable to simplify all techniques and equipment to increase reliability. But in order to perform some tasks at all during flight, a certain degree of complexity is unavoidable.

9.2 RECOMMENDATIONS

9.2.1 STUDIES ON PRESERVED SAMPLES

As has been mentioned several times, there are big gaps in our knowledge of how various constitutents of biological materials hold up under various forms of preservation. A broad laboratory study of this problem utilizing various types of preservation is necessary to determine the best methods for various constituents. Data on stability is meagre. All types of preservation have not been followed experimentally in similar ways so some of the data which is available cannot be compared. This is especially true for duration of reliable storage. The gaps concerning these points can only be filled by extensive experimental studies.

One particular area where there exists little, if any, information is comparison of the efficacy of various temperatures of frozen storage. Those few studies which have dealt with the stability of biological constituents in the frozen state have only dealt with a single temperature of storage (with the exception of freezing erythrocytes for later transfusion). Rate of freezing has also received little discussion aside from its importance in preserving blood cells intact. These two areas, freezing rate and storage temperature, require a great deal more study to permit the choice of optimum conditions for this type of preservation.

If samples are to be preserved and stored for post-flight analyses, studies should be started now to develop standard curves for stored body fluid constituents of interest. Preservation by chemical means, refrigeration, and freezing are the prime candidates. Samples of urine, feces, sweat, whole blood, plasma, and serum should be stored for periods of time not less than twice the planned resupply interval. Chemical preservatives which do not pose a crew safety hazard should be sought. Refrigeration at various temperatures should be tried, $4^{o}C$ seems to be a sacred number; whether $2^{o}C$ or $7^{o}C$ might be better is unknown. Freezing with controlled and uncontrolled rates of heat removal needs investigation as well as frozen storage at various temperatures. It must be emphasized that refrigerators and freezers made for home use, but which are routinely used in laboratories, are not satisfactory for these studies. Constant temperatures ($\pm$ 0.5 to $\pm$ 1.0^{o}C) should be maintained during storage, with a sensitive, precise, and accurate temperature sensor and recorder keeping track of temperature excursions.

9.2.2 LABORATORY PROCEDURES

Modifications of laboratory methods and equipment for easier use in weightlessness should be studied as soon as possible. Standardization of laboratory methods by the American Association of Clinical Chemists is a continuing process. The assistance of this and similar professional groups should be sought in an effort to ensure that laboratory procedures, whether on-board or performed post-flight, on preserved samples are as accurate, precise, and sensitive as possible.

The variation in test results among laboratories is too great to be ignored. Reference laboratories should participate in the planned experiments as a further check on accuracy and precision.

9.2.3 CREW SELECTION

The hypotheses that test pilots can be trained to perform experiments, and the converse, that scientists can be trained to fly the vehicle should be rigorously examined. The successful performance of tedious, sometimes odious, and occasionally complex laboratory procedures is totally dependent upon the crew. If the purpose of the planned missions is to obtain physiological and biochemical data, then highly motivated, well trained crews are essential.

Astronaut selection criteria should be re-examined and probably revised in order to obtain a crew possessing the mix of skills necessary for a top-notch laboratory.

Consideration should also be given to the inclusion of laboratory technicians in the crew. Many good scientists create the illusion that they have ten thumbs and two left hands when they work in a laboratory. The experiments for the AAP missions will be carefully planned with set schedules, thus requiring more doing than theorizing. A scientist-astronaut should be in charge of the laboratory and should make all major decisions, such as switching to an alternate schedule, omitting or adding a given procedure, etc.; but for routine tasks, and there will be many, a good technician would be preferable.

9.2.4 MATERIALS

Materials for use in a space laboratory must be chosen with care to avoid toxicity and/or fire hazards. A candidate list, from which materials for sample containers and handling equipment such as syringes, etc., can be fabricated, should be generated. These materials can then be tested for their applicability to laboratory usage. For example, the surface of plastics tends to be non-wettable. Surface etching may be required for tubes. The extent to which this changes the outgassing characteristics of the plastic is unknown.

9.2.5 SAFETY

During certain of the experiments, one or more of the astronauts will require some finite amount of time to disengage himself from apparatus (e.g., sweat test in Paragraph 3.3). Should an emergency arise occasioning the need to don a pressure suit, time will be of the essence. Procedures should be developed which will permit the astronaut to rid himself of encumbrances (an equipment design criterion must be quick release) in as short a period of time

as possible. Because of the array of equipment contemplated, studies to determine these emergency procedures should be done in parallel with equipment design.

Another hazard is presented by chemicals which may be preservatives or reagents. Each on-board laboratory procedure should undergo a risk assessment as a separate task after a preliminary definition of what equipments and procedures will be included in the operation of the orbiting laboratory.

9.2.6 THERMODYNAMIC PROPERTIES OF BIOLOGICAL FLUIDS

Either as a part of the long-term sample preservation studies mentioned above, or as a separate study, the thermodynamic properties of biological fluids need to be determined. Freezing of blood for transfusion use is largely an "overkill" technique. More heat removal capacity is available than is known to be needed. Overdesign, with safety factor added on to safety factor added on to margin of error, imposes severe weight, power, and volume penalties. Since these penalties have not been of primary concern in research and eleemosynary institutions, the major users of blood freezing and storing equipment, little definitive work on the thermodynamic properties of blood has been done. With knowledge, from studies on blood preservation, of just how cold blood must be for storage, how fast it must be cooled and then knowing how much heat has to be removed at what rate, this becomes an area where spin-off from the space program could influence the design of blood-banking equipment. Smaller, more efficient preservation units would sharply reduce their cost.

For urine, feces, sweat, and blood (including whole, plasma, and serum) information on thermal properties coupled with data on the effects of long term storage would be applicable to the shipment of biological fluids; specimens, when preserved and packaged properly, could be sent to reference laboratories at any time with assurance that the analytical results could be evaluated.

9.2.7 DEVELOPMENT OF FREON VAPOR CYCLE COOLING UNIT FOR ZERO-G OPERATION

The advantages of this type of cooling unit are discussed in Section 5. However, such equipment has not been developed for space vehicles. A study should be undertaken to determine development time and application to uses other than sample preservation.

9.2.8 INDIGENOUS MICROFLORA

Changes in both skin and intestinal microflora during confinement and isolation are under study in our laboratory. Sampling and recovery of microflora from the skin and feces needs considerable study in order to know what the original population is and how it changes with time during isolation and confinement. Since a pathogen can become the dominant microorganism, the health and safety of the crew will be endangered. With the time before long term missions starting to grow shorter, better methods of controlling the microflora must also be found. Additional studies on sampling and recovery of microorganisms and on control of the indegenous microflora are urgently needed.

9.2.9 THERMODYNAMIC ANALYSES OF SPATIAL HEAT SINKS (RADIATORS) FOR SPECIFIC SPACE FLIGHT MISSIONS

During the performance of this study, a lack of information on spatial heat sinks was noted. Weight, power, and volume penalties for given missions could possibly be reduced if the heat transfer requirements of each mission could be treated separately. A modular radiator design would permit addition to, or reduction of, cooling capacity as required and might allow more flexibility in mission planning. A feasibility study is recommended.

9.2.10 DEVELOPMENT OF A WASHING MACHINE FOR USE IN SPACE VEHICLES

A description of a washing machine concept is given in Section 6. As mission length increases, personal hygiene poses more of a problem. Aside from the possibilities of doing mineral balance studies, an on-board clothes washing capability may also result in a weight reduction since less extra clothing would have to be carried. It has been determined that such a washer is feasible; the next step should be design and fabrication of a prototype.

9.2.11 CENTRIFUGE DEVELOPMENT

Whether biochemical analyses are performed on-board, post-flight, or both, a centrifuge will be needed for the preparation of plasma and serum. A description of the centrifuge which is feasible is contained in Section 6. Design and fabrication of a prototype should be started promptly.

9.2.12 TRASH HANDLING

In the normal course of clinical laboratory operation a large amount of trash is generated. Dirty disposable syringes, needles, tubes, used agar plates replete with bacteria, bloody sponges, and the like all furnish excellent breeding grounds for more bacteria and fungi. In a space vehicle, sterilization and storage of compressed plastic appears to be a likely means of coping with this problem. A concept described in Section 6, and probably alternates, should be subjected to a design study in the near future because a trash handling system will be required in an orbiting laboratory.

9.2.13 EXPERIMENTAL DESIGN

The successful performance of contracting tasks depends in part upon the contractors' knowledge of the detailed rationale and experimental design of each experiment as further work related to experiments is initiated. Such information should, therefore, be made available to contractors.

9.2.14 EXPERIMENTER'S HANDBOOK

Most of the experiments have been proposed by principle investigators who have a good deal of scientific competence but little or no knowledge of the process by which hardware for these experiments is fabricated and qualified for flight. We urge NASA to fully exercise its management prerogatives and set forth a carefully defined method of handling experiment hardware (hardware is emphasized here because sample collection and handling analytical and preservation equipment are intimately associated with the experiments) design inputs. Our past experience indicates that the following criteria should be met by such a method:

a. Experimenter identity is recognized and retained throughout the program.

b. Experiment integrity is not compromised in the integration process.

c. NASA liaison will provide opportunity for experimenter-engineer crosstalk at both the contractor's facility and the experimenter's laboratory.

d. Contractor biomedical personnel should participate in experimenter-engineer meetings.

e. Established deadlines for experimenter design inputs.

f. Design inputs to be signed off by NASA and contractor personnel before prototype or flight design freeze.

g. Prototype hardware to be laboratory tested by both experimenters and contractor.

h. Design changes in flight hardware to be specified by experimenters within 90 days after receipt of prototype hardware.

i. Direct contact between contractor personnel and individual experimenters for resolution of problem areas.

Each of the criteria listed is based on GE's experience as an experiment (and experimenter) integrator. Very often the basic conflict between the hardware engineer and the academic research scientist is due simply to lack of understanding of the problems each must solve in order to perform an experiment within the constraints imposed by a space vehicle.

The importance of schedule deadlines must be emphasized. Program delays can occur if experimenters are not acutely aware that this is their program. Some items have a long lead time. Flight schedules can not be met if experimenter inputs are not made in a timely fashion. Design inputs should be signed off by both NASA and the contractor and engineering personnel so that the attainment of experimental objectives will be ensured and the contractor and NASA not faced with time-consuming, costly changes. Prototype testing should be performed in both the experimenter's laboratory and the contractor's laboratory so that design changes recommended for the flight articles will reflect desires tempered by reality.

The orientation of experimenters has, in the past, been hit or miss. That participation in such a costly venture, at no risk to the participant, is the case, has never fully been explained to the members of the scientific community who should be made aware, in detail, of the responsibilities of the experimenter.

To this end, we strongly recommend that an Experimenter's Handbook be prepared, including inputs from experimenters, engineers, and biologists with prior experience on experiment programs. Such a Handbook would explain, for example, why design changes increase in cost as the program moves along.

APPENDIX A

CLINICAL LABORATORY EVALUATIONS

PARAMETERS	SERUM OR PLASMA	URINE	WHOLE BLOOD	FECES	SWEAT	MICRO BIOLOGY
Creatine	X	X				
Creatinine	X	X				
Serum Proteins (electrophoresis)	X					
Mucoproteins & Related Biocolloids	X	X				
Sodium	X	X		X	X	
Potassium	X	X		X	X	
Chlorides	X	X		X	X	
Phosphates	X					
Alkaline Phosphatase	X					
Calcium	X	X		X	X	
Magnesium	X	X				
Manganese	X	X				
Bicarbonate	X					
Zinc	X	X				
Sulfates	X	X				
NPN	X					
BUN	X					
Uric Acid	X					
Glucose Tolerance			X			
Fat Tolerance		X				
Amino Nitrogen	X					
Total Nitrogen		X				
Blood Lactic Acid			X			
Bilirubin	X					
Standard Clinical Analysis		X				
Protein Bound Iodine (PBI)	X					
17-Hydroxy Corticosteroids		X				
Catecholamines	X					
Thyroxine	X					
Thyroxine Binding Prealbumin (TBPA)	X					

PARAMETERS	SERUM OR PLASMA	URINE	WHOLE BLOOD	FECES	SWEAT	MICRO BIOLOGY
Aldosterone		X				
Antidiuretic Hormone (ADH)	X	X				
Adreno-Corticotrophic Hormone (ACTH)	X					
Serotonin		X				
Specific Gravity		X				
Proteins		X				
pH		X				
Hematocrit			X			
Reticulocyte Count			X			
RBC (total)			X			
WBC (total)			X			
WBC Differential			X			
RBC Cell Mass (Isotopes)			X			
RBC Survival			X			
Hemoglobin			X			
Platelet Count			X			
Plasma Volume ($RISA^{125}$)	X					
WBC Motility and Phagocytic Activity						
Platelet Estimate			X			
Platelet Adhesiveness			X			
Fibrinogen	X					
Fibrinolytic Activity	X					
Prothrombin Activity	X					
Plasma Thromboplastic Component (PTC)	X					
Antihemophylic Globulin (AHG)	X					
Immunoglobulins (complement and antibodies)			X			
Cytogenic Studies of Lymphocytes (karyotyping)			X			
Clotting Time			X			
Clot Retraction			X			
Sampling and Culturing of Body Flora						X

PARAMETERS	SERUM OR PLASMA	URINE	WHOLE BLOOD	FECES	SWEAT	MICRO BIOLOGY
Colony Counts						X
Microbiological Identifications						X
LDH Isozymes	X					
Immune Bodies	X					
Transferins	X					
Hemoglobin			X			
Methemoglobin			X			

APPENDIX B

PRELIMINARY DESIGN FEATURES

For purpose of this contract, preliminary design figures are given below. These figures are approximations and do not represent any specific mission configuration. The figures provided apply to the total of all medical experiments. As no data applicable to the specific equipment considered in this work statement can be supplied at this time, this information can serve only as a general design guide.

Volume available for medical experiments	100 $ft.^3$
Pressure	5 psia
Gas Composition	Pure Oxygen, or Oxygen plus Nitrogen
Total allowable weight for medical experiments	250 lbs.
Power = 29 $\pm$ 2 d.c. and 3 phase 400 cps a.c.	500 watts for medical experiments
Temperature	$75^{o}F \pm 10^{o}F$
Relative Humidity	40-70%
Heat Removal Capacity (Medical Experiments)	
Airlock permits extra vehicular activity without depressurization	
Radiation	4 $mr/ft^2/day$
Data Transmission (Medical Experiments)	10k bits/sec. of PCM telemetry
Acceleration	20 G's maximum
Vibration	0.06 G's (100 - 500 cps) 0.1 G's (100 - 200 cps) 0.3 G's(15 - 32 cps)

APPENDIX C

General Guideline Design Information

(From NASA RFP 10-1243, Integrated Medical and Behavioral Laboratory Measurement System)

Maximum Experiment Equipment	100 ft^3
Pressure	5 psia
Gas Composition	Pure Oxygen, or Oxygen plus Nitrogen
Total allowable weight for medical experiment equipment	250 lbs.
Power = 29 $\pm$ 2 d.c. and 3 phase 400 cps a.c.	1000 watts for medical experiments
Temperature	$75^{o} \pm 15^{o}F$
Relative Humidity	40-70%
Radiation	4 mr/ft^2/day
Data Transmission (Medical Experiment Equipment)	10 K bits/sec of PCM telemetry
Acceleration	7 G's longitudinal
Vibration	0.02 G^2/cps at 20 cps and Linear to 0.09 G^2/cps at 400 cps Constant at 0.09 G^2/cps to 800 cps

Dimensions for Storage Module Sizing

Annex C, p. 11, Section 2.3.1 Command Module

30-inch diameter tunnel for egress from spacecraft during flight (possibility of using this hatch to move modules)

Annex C, p. 30, Section 2.4.3 The Airlock and The S-IVB Modifications

Airlock - cylinder with 65-inch diameter and 16-foot length

Airlock EVA exit and S-IVB manhole are approximately 43 inches in diameter